Saudubray
van den Berghe
Walter

Inborn Metabolic Diseases

Diagnosis and Treatment

5th Edition

Springer

Jean-Marie Saudubray

Georges van den Berghe

John H. Walter (Editors)

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With 87 Figures

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Preface to the 5th edition

Inborn Metabolic Diseases: Diagnosis and Treatment has for many years been considered a classic textbook, indispensable to those involved in the care of patients with inborn errors of metabolism, including paediatricians, clinical biochemists, neurologists, specialists in internal medicine, geneticists, dietitians, nurses and clinical psychologists. The 4th edition was published five years ago and sadly, since then, in 2009, John Fernandes, the early spiritual father, of the book, has died.

This new 5th edition has been extensively revised, but remains focused on the clinical symptoms and signs at presentation, the investigations necessary to reach a correct diagnosis and the management of specific disorders. We have also borne in mind the need to keep the book to a reasonable size. Two new chapters have been added; the first on the diagnostic approach to neurometabolic disorders in adulthood and the second on disorders of phospholipid and sphingolipid synthesis. All chapters from the 4th edition have been carefully updated, and some considerably extended; for example, the chapter on the transport of metals now includes iron, manganese and selenium transport disorders in addition to those of copper, zinc and magnesium. Chapter 1 has also been extended and now includes many genetic disorders affecting transporters, channels and enzymes implicated in the logistics and regulation of the cells, disorders which exemplify the bridge between classic metabolic diseases and those caused by structural proteins mutations.

As with the previous edition the book can be used in two main ways:
If the diagnosis is not known the reader should first refer to Chapter 1 for disorders in paediatric patients or Chapter 2 for those in adults. These chapters, in which there are a number of algorithms and tables, list the clinical findings. In Chapter 1 there are five main sections, dealing with (1) symptoms in the antenatal period; (2) symptoms in the neonatal period and early infancy; (3) acute presentations in late infancy and beyond; (4) chronic and progressive neurodegeneration; and (5) specific organ/system involvement. In this edition the sections on neuroradiology and neurophysiology have been developed further. In both tables and text, those disorders for which treatment is available are printed in bold so they can be recognised at a glance.

In Chapter 2 a comprehensive approach to neurometabolic disorders is given for all classic neurological syndromes.

If the diagnosis is already suspected or is indicated from reference to Chapters 1 or 2, the reader can then go directly to the relevant chapter to obtain more specific information. To simplify this process each of these chapters is presented in a uniform format.

As before, we continue to advocate referral to specialist centres for 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 and South American countries comparable lists are compiled by the American (SIMD), Japanese (JIMD), Australian (AIMD) and South Latin America (SLEIMPN) societies for the study of inherited metabolic diseases, respectively.

The editors welcome new authors of old and new chapters and pay tribute to those authors who, while not involved this time, laid the foundation for this book.

Jean-Marie Saudubray
Georges van den Berghe
John H. Walter
Autumn 2011

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Abbreviation List

AA	Aminoacidopathies
AAC	Amino acid chromatography
ACAD	AcylCoA dehydrogenase
AGAT	L-Arginine:glycine amidino transferase
AMACR	α-methyl-acyl-CoA racemase
APGBD	Adult Polyglucosan Body Disease
ASL	Argininosuccinate lyase
ASS	Argininosuccinate synthetase
BBGD	Biotin-responsive basal ganglia disease
CAT	Carnitine-acylcarnitine translocase
CBS	Cystathionine-β-synthase
CPS	carbamoylphosphate synthetase
CDG	Congenital disorders of glycosylation
Cbl	Cobalamin
CIT	Citrullinemia
CPS	Carbamoyl phosphate synthetase
CPT	Carnitine palmitoyl transferase
CTX	Cerebrotendinous xanthomatosis
ETF	Electron transfer flavoprotein
ETFDH	Electron transfer flavoprotein dehydrogenase
FBP	Fructose-1,6-bisphosphatase
GA	Glutaric aciduria
GAL	Galactose/Galactosemia
GAMT	Guanidinoacetate *N*-methyltransferase
GSD	Glycogen storage diseases (glycogenoses)
GCMS	Gas chromatography mass spectrometry
GLUT	Glucose transporter
GTP	Guanosine triphosphate
GTPCH	Guanosine triphosphate cyclohydrolase
G6Pase	Glucose-6-phosphatase
HCYS	Homocystinuria
HFI	Hereditary fructose intolerance
HI	Hyperinsulinism
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
Homocysteine RD	Homocysteine remethylation defects
IVA	Isovaleric acidaemia
FAO	Fatty acid oxidation
FAOD	Fatty acid oxidation defects
KB	Ketone bodies
Lac	Lactate
LCAD	Long-chain acyl-CoA dehydrogenase
LCHAD	Long-chain hydroxyacyl-CoA dehydrogenase
LPI	Lysinuric protein intolerance
MAD	Multiple acyl-CoA dehydrogenase
MCAD	Medium-chain acyl-CoA dehydrogenase deficiency
MCD	Multiple carboxylase deficiency
MELAS	Mitochondrial encephalomyopathy lactic acidosis, and stroke-like episodes
MLD	Metachromatic leukodystrophy
MMA	Methyl malonic aciduria
MNGIE	Mitochondrial Neuro GastroIntestinal Encephalomyopathy
MPS	Mucopolysaccharidosis
MSUD	Maple syrup urine disease
MTHFR	Methylene tetrahydrofolate reductase
NKH	Nonketotic hyperglycinaemia
OA	Organic acidurias/emias
OAC	Organic acid chromatography
OAT	Ornithine amino transferase
OCT	Ornithine carbamoyl transferase
OXPHOS	Oxidative phosphorylation
PA	Propionic acidaemia / aciduria
PANK2	Panthothenate kinase 2
PBD	Peroxisome biogenesis defects
PC	Pyruvate carboxylase
PDH	Pyruvate dehydrogenase
PEX	Peroxin
PKU	Phenylketonuria
PLA2G6	Calcium-independent phospholipase A2
3 PGD	3-phosphoglycerate dehydrogenase
PNPO	Pyridoxamine-5-phosphate oxidase
PTP	Phospholipid transfer protein
Pyr	Pyruvate
RCD	Respiratory chain disorder
RD	Respiratory chain defect
SCAD	Short chain acyl-CoA dehydrogenase
SLC	Thiamine transporter
SCHAD	Short-chain hydroxyacyl-CoA dehydrogenase
SUCCLA 2	Succinyl CoA synthetase
SO	Sulfite oxidase
SSADH	Succinic semialdehyde dehydrogenase
TALDO	Transaldolase
Tandem MS/MS	Tandem mass spectrometry
Triple H	hyperammonaemia, hyperornithinaemia, homocitrullinuria
TYR I and II	Tyrosinemia type II and II
UCD	Urea cycle defect
VLCAD	Very-long-chain acyl-CoA dehydrogenase
VLCFA	Very-long-chain fatty acids

I Diagnosis and Treatment: General Principles

Clinical Approach to Inborn Errors of Metabolism in Paediatrics

Jean-Marie Saudubray

J.-M. Saudubray et. al (Eds.), *Inborn Metabolic Diseases,* DOI 10.1007/978-3-642-15720-2_1,

Inborn errors of metabolism (IEM) are individually rare, but collectively numerous. The recent 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, too expensive or unreliable and, as a consequence, a simple method of clinical screening is mandatory before the initiation of 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 that symptoms that persist and remain unexplained after the initial treatment and after the usual investigations for more common disorders have been performed 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. Carefully review all autopsy findings.
- Do not confuse a symptom or a syndrome with aetiology – the underlying cause may be an IEM that has yet to be defined.
- Remember that an IEM can present at any age, from fetal life to old age.
- Be aware that although most genetic metabolic errors are hereditary and transmitted as recessive disorders, the majority of individual cases appear sporadic.
- Initially consider inborn errors that are amenable to treatment, particularly in patients who are acutely unwell.
- Obtain help from specialised centres.

Do not miss a treatable disorder.
Provide care for the patient first (emergency treatment) and then for 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 that Give Rise to Intoxication. This group includes inborn errors of intermediary metabolism that lead to an acute or progressive intoxication from the accumulation of toxic compounds proximal to the metabolic block. It is in this group that the inborn errors of amino acid catabolism (phenylketonuria, maple syrup urine disease, homocystinuria, tyrosinaemia, etc.), most organic acidurias (methylmalonic, propionic, isovaleric etc.), congenital urea cycle defects, sugar intolerances (galactosaemia, hereditary fructose intolerance), metal intoxication (Wilson, Menkes, haemochromatosis) and porphyrias belong. All the conditions in this group share clinical similarities: they do not interfere with the embryofetal development and 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 amino acid, organic acid and acylcarnitine chromatography. Most of these disorders are treatable and require the emergency removal of the toxin by special diets, extracorporeal 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 amino acid synthesis (serine, glutamine, and proline / ornithine) can also be included in this group, since they share many characteristics: they are inborn errors of intermediary metabolism, their diagnosis relies on plasma, urine and CSF investigations (amino acid, organic acid analyses, etc.) and some are amenable to treatment even when the disorder starts in utero, for example 3-phosphoglycerate dehydrogenase deficiency [1].

Group 2: Disorders Involving Energy Metabolism. These consist of IEM with symptoms due at least partly to a deficiency in energy production or utilisation 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 acidaemias (defects of pyruvate transporter, pyruvate carboxylase, pyruvate dehydrogenase and the Krebs cycle enzymes), mitochondrial respiratory chain disorders (disturbing the respiratory chain itself, mitochondrial transporters, or the synthesis of coenzyme Q10) and the defects of fatty acid oxidation and ketone body metabolism. Only the last and coenzyme Q10 defects are partly treatable. 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 (some of which are treatable) and inborn errors of the pentose phosphate pathways (untreatable). Common symptoms in this group include hypoglycaemia, hyperlactataemia, hepatomegaly,

severe generalised 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 defects can interfere with the embryofetal development and give rise to dysmorphism, dysplasia and malformations [2]. Diagnosis is difficult and relies on function tests, enzymatic analyses requiring biopsies or cell culture and molecular analyses.

Group 3: Disorders Involving Complex Molecules. This group involves cellular organelles and includes diseases that disturb the synthesis or the catabolism of complex molecules. Symptoms are permanent, progressive, independent of intercurrent events and unrelated to food intake. All lysosomal storage disorders, peroxisomal disorders, disorders of intracellular trafficking and processing, such as alpha-1-antitrypsin, congenital disorders of glycosylation (CDGs), and inborn errors of cholesterol synthesis belong to this group. Many other defects disturbing various systems and implicated in complex molecules processing can be hypothesised, as illustrated by the recently described CEDNIK syndrome (due to a mutation in the *SNAP 29* gene coding for a SNARE protein involved in intracellular vesicle function [3]) or infantile neuroaxonal dystrophy (due to mutations in the *PLA2G6* gene coding for a phospholipase A2 catalysing the release of fatty acids from phospholipids (▶ Chapter 35). These last disorders cannot be detected by classic biochemical methods and require DNA analysis for their recognition, making the bridge between IEM and genetic disorders involving structural proteins.

Almost none of these disorders are treatable acutely; however, enzyme replacement therapy is now available for several lysosomal disorders.

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 organ failure.
- Chronic and progressive neurological symptoms (developmental delay, mental retardation, epilepsy, neurological deterioration and psychiatric signs).
- Specific and permanent organ/system presentations that may concern all medical specialities (cardiology, dermatology, endocrinology, gastroenterology, haematology, etc.).

1.2 Antenatal Symptoms (◘ Table 1.1–Table 1.3)

These can be classified into three major clinical categories: (1) true malformations (e.g. skeletal malformations,

◘ **Table 1.1.** Antenatal symptoms according to the physiopathological classification of inborn errors of metabolism

Group	Disorders	None	Functional	Dysplasia	Malformations
I	AA/AO catabolism	X	-	-	-
	Porphyria/metals	-	X	-	-
	AA synthesis	-	X	X	X
II	Glycolysis	X	-	-	-
	Pentose-P	-	X	-	-
	FAO	-(X)	(X)	(X)	
	Respiratory chain	-	X	X	(X)
III	Lysosome	-	X	X	-
	Peroxisome	-	X	X	-
	N-Glycoxylation	-	X	X	-
	O-Glycosylation	-	X	X	X
	Cholesterol	-	X	X	X

P, phosphate; ***X*, frequently to always present; (*X*), rarely observed**

Table 1.2. Inborn errors of metabolism with coarse facies or intrauterine growth retardation and their differential diagnosis

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 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	
Alcoholic fetal syndrome Infants born to mothers with untreated phenylketonuria Cholesterol biosynthesis defects CDG 1n Lysosomal storage disorders Many nonmetabolic polymalformative syndromes Peroxisomal disorders Respiratory chain disorders Transaldolase deficiency	

congenital heart disease,visceral aplasias and neural tube defects); (2) dysplasias (e.g. cortical heterotopias, cortical cysts, posterior fossa abnormalities, polycystic kidneys, liver cysts); and (3) functional manifestations (intrauterine growth retardation, hydrops fetalis, hepatosplenomegaly and microcephaly). According to this classification, true irreversible malformations are only observed in *O*-glycosylation disorders, in cholesterol synthesis defects, in glutamine synthetase deficiency (lissencephaly) and, rarely, in glutaric aciduria type II and in respiratory chain disorders (Table 1.1, Table 1.3). It should be noted that the congenital microcephaly observed in serine synthesis defects is partly reversible on early treatment, as in a functional abnormality. Lysosomal, peroxysomal and *N*-glycosylation defects are responsible for dysplasia and functional abnormalities that are reversible to greater or lesser degrees. The vast majority of 'true intoxication' disorders (amino acid and organic acid catabolism defects) do not disrupt normal embryofetal development and consequently are not associated with dysmorphism or antenatal manifestations (Table 1.1). Coarse facies is present in many lysosomal disorders and is an important diagnostic sign (Table 1.2). Untreated maternal disturbances (e.g. PKU) can be responsible for fetal dysplasia (Table 1.3).

1.3 Symptoms in the Neonatal Period and Early Infancy (<1 year) [4, 5]

1.3.1 Clinical Presentations

The neonate has a limited repertoire of responses to severe illness. IEM may present with nonspecific manifestations such as respiratory distress, hypotonia, poor sucking reflex, vomiting, diarrhoea, dehydration, lethargy and 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 hae-

Table 1.3. Inborn errors of metabolism with dysplasia, dysmorphism and malformations

Dysplasia, dysmorphism	
Maternal metabolic disturbances	**PKU** (dysmaturity, heart defect, microcephaly, special face, hypotrophy) Alcohol (dysmorphia, hypotrophy) Tyrosinaemia type II **Diabetes** (macrosomia) Drugs (dysmorphia, hypotrophy) Vitamin deficiencies (riboflavin)
Inborn errors affecting the fetus	Carnitine palmityl transferase II deficiency (with renal cysts) Chondrodysplasia punctata (calcific stippling of epiphyses, coronal clefts of vertebral bodies) D-2-Hydroxyglutaric aciduria Glutaric aciduria II (MADD) (with renal cysts, brain dysplasia) Inborn errors of collagen metabolism **Hyperinsulinism** (macrosomia, dysmorphism) Hypoparathyroidism Hypophosphatasia Leprechaunism Lysosomal storage disorders (hydrops fetalis and dysostosis multiplex) Mevalonate kinase deficiency (dysmorphism) Mitochondrial glutamate transporter defect (abnormal corpus callosum and gyration of temporo-parietal regions) Peroxisomal biogenesis defects (renal cysts,facial dysmorphism, neuronal migration disorder, germinolytic cysts) **Pyruvate dehydrogenase deficiency** (brain dysgenesis, agenesis of the corpus callosum) Respiratory chain defects Transaldolase deficiency (hydrops fetalis)
Malformations	
Cholesterol synthesis defects (affecting developmental signalling pathways) *O*-Glycosylation and related defects Glutamine synthetase deficiency (lissencephaly) Respiratory chain defects (ventricular septal, vertebral and limb defects, VACTERL association) 3-OH-isobutyryl CoA deacylase deficiency (limbs, vertebrae) Nonketotic hyperglycinaemia (dysgenesis of the corpus callosum and gyral malformations)	

morrhage, and it is important to make a critical review of 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 entirely 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, bacteriological studies and cerebral ultrasound, generally give 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 nonspecific manifestations of neonatal hypoxia, infection or other common diagnoses take on a new significance. In energy deficiencies (group 2 disorders), the clinical presentation is often less suggestive 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 in the following conditions.

Neurological Deterioration (Coma, Lethargy)

Most inborn errors that result in intoxication or energy deficiency are brought to medical attention because of neurological deterioration. In the case of 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.

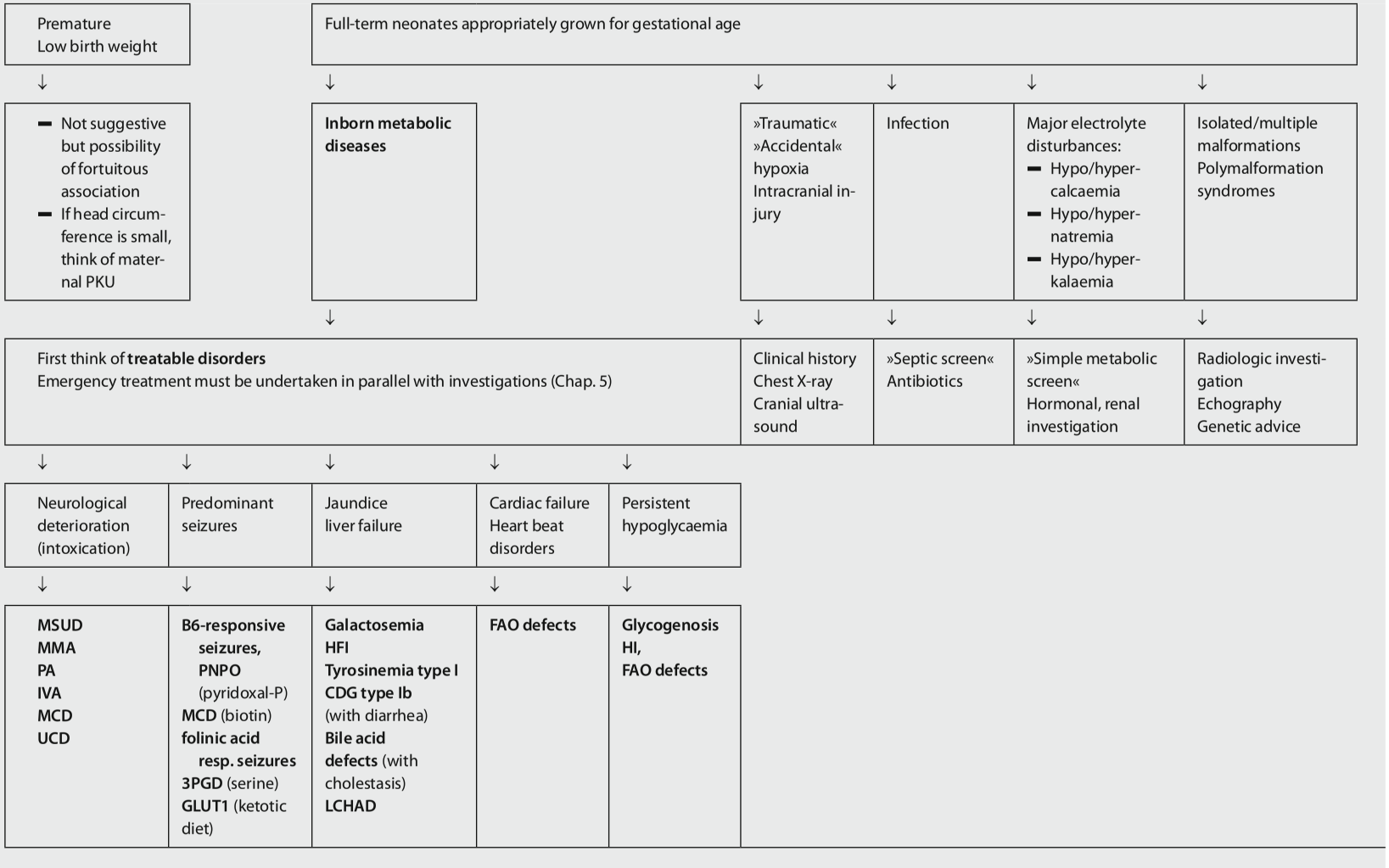

Fig. 1.1. The 'sick' neonate: an algorithm for screening for treatable inborn errors of metabolism

At a more advanced state, neurovegetative problems with respiratory abnormalities, hiccups, apnoeas, bradycardia and hypothermia can develop. In the comatose state, characteristic changes in muscle tone and involuntary movements appear. In **maple syrup urine disease** (**MSUD**) generalised hypertonic episodes with opisthotonus are frequent, and boxing or pedalling movements and also slow limb elevations, spontaneous or upon stimulation, are observed. Conversely, most nonmetabolic 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 **organic acidurias** 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 odour is present in some diseases in which volatile metabolites accumulate; the most important examples are the maple syrup odour of **MSUD** and the sweaty feet odour of **isovaleric acidaemia** (**IVA**) and **type II glutaric acidaemia**. 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 suggestive and varies more widely in severity. In many conditions there is no symptom-free interval. The most frequent findings are a severe generalised hypotonia, rapidly progressive neurological deterioration and possible dysmorphism or malformations. However, in contrast to the intoxication group, lethargy and coma are rarely initial signs. Hyperlactataemia with or without metabolic acidosis is very frequent. Cardiac and hepatic involvement are commonly associated (► below).

Only a few lysosomal storage disorders present in the neonatal period with neurological deterioration. By contrast, most peroxisomal biogenesis defects present at birth with dysmorphism and severe neurological dysfunction. A severe neurological disease with vitamin K deficiency, intracranial bleedings, seizures and death in infancy has recently been described in CDG-IIL; this is caused by *COG6* mutations.

▪ Seizures

Always consider the possibility of an IEM in a neonate with unexplained and refractory epilepsy [6, 7]. Four treatable metabolic disorders can present in the neonatal period or early in infancy predominantly with 'intractable' seizures: **pyridoxine-responsive seizures**, **pyridox(am)ine-5′-phosphate oxidase deficiency** (responsive to pyridoxal phosphate but not to pyridoxine), **3-phosphoglycerate dehydrogenase deficiency** and **other inborn errors of serine synthesis** [8] (responsive to serine supplementation) and **persistent hyperinsulinaemic hypoglycaemia**. **Folinic acid-responsive epilepsy** is probably not a true entity, but rather corresponds to undiagnosed B_6-responsive seizures [9]. Biotin-responsive **holocarboxylase synthetase deficiency** can also, albeit rarely, present predominantly with neonatal seizures. **GLUT1 deficiency** (brain glucose transporter), which can be treated with hyperketotic diet, and biotin-responsive **biotinidase deficiency** can also present in the first months of life as epileptic encephalopathies.

Many other nontreatable inherited disorders can present in the neonatal period or early in infancy with severe epilepsy: nonketotic hyperglycinaemia, d-glyceric aciduria, mitochondrial glutamate transporter defect, GABA transaminase deficiency, glutamine synthetase deficiency (all presenting with early myoclonic encephalopathy with or without a burst-suppression EEG pattern), peroxisomal biogenesis defects, respiratory chain disorders, sulfite oxidase deficiency, defects of purine metabolism, CDGs and Menkes disease. In all these conditions epilepsy is severe, with an early onset, and can present with spasms, myoclonus and partial or generalised tonic / clonic crises.

▪ Hypotonia

Severe hypotonia is a common symptom in sick neonates. It is generally observed in nonmetabolic inherited diseases (mainly in severe fetal neuromuscular disorders). Only a few inborn errors of metabolism present with isolated hypotonia in the neonatal period, and 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 hyperlactataemia, respiratory chain disorders, urea cycle defects, NKH, sulfite oxidase (SO) deficiency, peroxisomal disorders and trifunctional enzyme deficiency. Central hypotonia is associated with lethargy, coma, seizures and neurological symptoms in NKH, SO deficiency and peroxisomal disorders. Central hypotonia with characteristic metabolic changes is also observed in congenital lactic acidosis and **urea cycle disorders** (hyperammonaemia). Severe forms of Pompe disease (alpha glucosidase deficiency) and fatal congenital heart glycogenosis due to mutation in *PRKAG2* gene [10] can initially mimic respiratory chain disorders, or trifunctional enzyme deficiency when generalised hypotonia is associated with cardiomyopathy. However, Pompe disease does not, strictly speaking, manifest in the neonatal period. Finally, one of the most frequent causes of neonatal hypotonia is Prader-Willi syndrome, where central hypotonia is apparently an isolated symptom at birth. The latter syndrome is mimicked by the hypotonia cystinuria syndrome, a recently described autosomal

recessive disorder that is due to a deletion of two 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 [11]. The newly described riboflavin transporter 2 defects present early in infancy with hypotonia and respiratory failure (Brown-Vialetto-van Laere syndrome) [12] (► Chapter 13).

The three neurological presentations are summarised in ◘ Table 1.4.

▪ Hepatic Presentation

Four main clinical groups can be identified:

- Massive hepatomegaly with hypoglycaemia and seizures suggest **glycogenosis type I or III**, or **gluconeogenesis defects**. **Severe hyperinsulinism** can sometimes be associated with moderate hepatomegaly.
- Liver failure (jaundice, coagulopathy, hepatocellular necrosis with elevated transaminases,hypoglycaemia, ascites and oedema) in the 1st week of life suggests neonatal haemochromatosis, respiratory chain disorders (mostly mitochondrial DNA depletion), transaldolase deficiency (which can present with hydrops fetalis), **galactosaemia, hereditary fructose intolerance** (now very rare since infant formulas are fructose free) and **tyrosinaemia type I** (after 3 weeks). GRACILE syndrome [13, 14] displays severe fetal growth retardation, lactic acidosis, failure to thrive, hyperaminoaciduria, very high serum ferritin, haemosiderosis of the liver and early death. In addition, severe, but reversible, hepatocellular necrosis and acute hepatitis-like episodes have recently been reported in HHH syndrome (► Chapter 22) and which should therefore be added to the list of metabolic disorders causing liver failure.
- Cholestatic jaundice with failure to thrive is a predominant finding in alpha-1-antitrypsin deficiency, Byler disease, **inborn errors of bile acid metabolism**, peroxisomal disorders, Niemann-Pick type C disease, CDGs (**mostly type 1b**), and citrin deficiency [15, 16]. Cerebrotendinous xanthomatosis, citrin deficiency, Niemann-Pick C and hereditary spastic paraparesis type V due to oxysterol 7-α hydroxylase defect can present as a transient asymptomatic jaundice before neurological signs appear later in life. Hepatic presentations of inherited fatty acid oxidation (FAO) disorders and urea cycle defects consist in 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. It must be emphasised that there are frequent difficulties in investigating patients with severe hepatic failure. At an advanced stage of hepatocellular damage many nonspecific abnormalities can be present, including mellituria (galactosuria, glycosuria, fructosuria), hyperammonaemia, hyperlactataemia, hypoglycaemia after a short fast, hypertyrosinaemia (>200 μmol/l) and hypermethioninaemia (sometimes higher than 500 μmol/l).
- Hepatosplenomegaly (HSM) with signs of storage (coarse facies, macroglossia, hydrops fetalis, ascites, oedema, dysostosis multiplex, vacuolated lymphocytes) are seen in lysosomal disorders (GM1 gangliosidosis, sialidosis type II, I-cell disease, Niemann-Pick A, MPS type VII, galactosialidosis), CDG Ik [17] and congenital erythropoietic porphyria. HSM with inflammatory syndrome and haematological or immunological features can be seen in lysinuric protein intolerance (macrophage-activating syndrome, leukopenia), mevalonic aciduria (inflammatory syndrome and recurrent severe anaemia) and transaldolase deficiency (hydrops fetalis with severe anaemia).

▪ Cardiac Presentation

Some metabolic disorders can present predominantly with cardiac disease. Cardiac failure and a dilated hypertrophic cardiomyopathy (pure dilated cardiomyopathy is 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 EKG and vacuolated lymphocytes) or fatal congenital heart glycogenosis due to mutations in the *PRKAG2* gene [10]. Methylglutaconic aciduria is found in Barth syndrome and ketoglutarate excretion in ketoglutarate dehydrogenase deficiency. Some respiratory chain disorders appear to be tissue specific and are only expressed in the myocardium [18], while many others are ubiquitous, such as the recently described mitochondrial translation elongation factor defect [19]. Cardiomyopathy and long QT syndrome are frequent complications of severe forms of propionic acidaemia.

CDG type Ia can sometimes present in infancy with cardiac failure due to pericardial effusions, cardiac tamponade and cardiomyopathy. Many defects of long-chain fatty acid oxidation can present with cardiomyopathy and/or arrhythmias and conduction defects (auriculoventricular block, bundle branch blocks, ventricular tachycardia), which may lead to cardiac arrest.

Table 1.4. Neurological presentations

Predominant clinical presentation	Main clinical features	Biological signs	Possible diagnoses
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 odour	Ketosis, acidosis	**MSUD** (odour)
		Hyperlactataemia Leukoneutropenia Thrombopenia Hyperammonaemia Characteristic changes of AAC or OAC	**MMA, PA, IVA** (odour) **MCD** **Urea cycle defects** **GA type II** (odour)
Seizures: sometimes metabolic, sometimes treatable	Isolated or generalised	Metabolic ketoacidosis Organic acid profile	**MCD**
		None	**Pyridoxine-responsive seizures**
		Hypocalcaemia Hypomagnesaemia	**Congenital magnesium malabsorption**
		Severe hypoglycaemia	**HI**
	West syndrome Hypsarrhythmia Severe microcephaly	Low serine (plasma / CSF)	**3PGD deficiency**
		Low copper	Menkes disease
	Severe hypotonia Myoclonic jerks Burst-suppression	Low HVA, 5HIAA in CSF Vanillactic acid (urine) Hyperglycinaemia	**PNPO** (pyridoxal phosphate responsive) NKH, GABA transaminase deficiency
		None	Glutamate transporter defect
		Increased urine sulfite and *S*-sulfocysteine (AAC)	Sulfite oxidase deficiency
	Facial dysmorphia Malformations Severe hypotonia	VLCFA, phytanic, plasmalogen	Peroxisomal defects
		Glycosylated transferrin	CDG
		Sterols in plasma	Cholesterol biosynthesis defects
Severe hypotonia, rarely metabolic	Isolated	None Massive cystinuria	Prader-Willi syndrome Hypotonia/cystinuria
Not treatable	Fetal distress Hydramnios Arthrogryposis Respiratory failure	None	Severe fetal neuromuscular diseases Steinert disease Myasthenia Congenital myopathy Sensitivomotor neuropathy
		Hyperlactataemia	Respiratory chain disorders
		Acylcarnitine and organic acid profile	**Riboflavin transporter 2 defect**
	Predominant dysmorphia Malformations	VLCFA, phytanic plasmalogen Sterols in plasma Tubulopathy Glycosylated transferring APO-B glycosylation Chromosome analyses	Peroxisomal defects Cholesterol metabolism defects Lowe disease Glycosylation defects *O*-Glycosylation defects Polymalformative syndromes with muscular dystrophy (Walker Warburg, etc.) Chromosomal abnormalities
	Cataract Tubulopathy	Hyperlactataemia Enzyme/DNA analyses	Lowe syndrome Respiratory chain disorders
	Cardiomyopathy Macroglossia	Vacuolated lymphocytes Hyperlactataemia Acylcarnitine	**Pompe disease** Cardiac glycogenosis due to *PRKAG2* mutation Respiratory chain disorders Trifunctional enzyme deficiency

5HIAA: 5-hydroxyindoleacetic acid; *HVA*, homovanillic acid. Treatable disorders are shown in **boldface type**

1.3.2 Metabolic Derangements and Diagnostic Tests

▪ Initial Approach and Protocol for Investigation

As soon as there is a clinical suspicion of an IEM, general supportive measures and laboratory investigations should be undertaken concurrently (◘ Table 1.5). Abnormal urine odours 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 ketoacids such as occur in MSUD. However, it has now largely been abandoned because of its poor specificity and because amino acid chromatography has become much more readily available. Hypocalcaemia 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 organic acidurias is usually accompanied by an elevated anion gap (the difference between plasma sodium and the sum of chloride and bicarbonate). Urine pH should be below 5; otherwise, renal acidosis must be considered. Metabolic acidosis resulting from IEM may develop as a result of the accumulation of fixed anions (lactate, ketone bodies), an organic acid or a combination of both or of loss of bicarbonate, which is usually due to tubular dysfunction. In metabolic acidosis resulting from a 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 is generally normal (i.e. 10-15 mmol/l). In metabolic acidosis with a wide anion gap the presence or absence of ketonuria is the major clinical clue to the diagnosis.

A normal blood pH does not exclude hyperlactataemia, as neutrality is usually maintained until serum levels reach 6 mmol/l (as long as bicarbonate level remains >18 mmol/). Ammonia and lactic acid should be determined in all newborns at risk. An elevated ammonia level in

◘ **Table 1.5.** Protocol for emergency investigations

	Immediate investigations	Storage of samples
Urines	Smell Look Acetone (Acetest, Ames) Reducing substances (Clinitest, Ames) Ketoacids (DNPH) pH (pHstix Merck) Sulfitest (Merck) Electrolytes (Na, K), urea, creatinine Uric acid	Urine collection: collect fresh sample and put it in the refrigerator Freezing: freeze samples collected before and after treatment at –20°C and collect an aliquot 24 h after treatment. Do not use them without having expert metabolic advice Metabolic investigations: OAC, AAC, orotic acid, porphyrins
Blood	Blood cell count Electrolytes (calculate anion gap) Glucose, calcium Blood gases (pH, pCO_2, HCO_3, pO_2) Uric acid Prothrombin time Transaminases (and other liver tests) Ammonia Lactic, pyruvic acids 3-Hydroxybutyrate, acetoacetate Free fatty acids	Plasma (5 ml) heparinised at -20°C Blood on filter paper: 2 spots (as for Guthrie test) Whole blood (10-15 ml) collected on EDTA and frozen (for molecular biology studies) Major metabolic investigations: total homocysteine, AAC, acylcarnitine (tandem MS), OAC, porphyrins, neurotransmitters (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)

CSF, Cerebrospinal fluid; *DNPH*, dinitrophenylhydrazine; *ECG*, electrocardiogram; *EDTA*, ethylenediaminetetra-acetic acid; *EEG*, electroencephalogram

itself can induce respiratory alkalosis; hyperammonaemia with ketoacidosis suggests an underlying organic acidaemia (OA), but hyperammonaemia can also occur in isolation. Elevated lactic acid levels in the absence of infection or tissue hypoxia are a significant finding. Moderate elevations (3-6 mmol/l) are often observed in OAs and in the hyperammonaemias; levels greater than 10 mmol/l are frequent in hypoxia (► Section 1.4.2). Propionic, methylmalonic and isovaleric acidaemias may induce granulocytopenia and thrombocytopenia, which may be mistaken for sepsis. Transaldolase deficiency and early-onset forms of mevalonate kinase deficiency present with severe recurrent haemolytic anaemia.

The storage of adequate amounts of plasma, urine, blood on filter paper, and CSF is an important element in reaching a diagnosis. The utilisation of these valuable 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 1.6. Classification of inborn errors revealed in the neonatal period and early in infancy

Types	Clinical type	Acidosis/ ketosis	Other signs	Most usual diagnosis (disorder/enzyme deficiency)	Elective methods of investigation
I	**Neurological deterioration**, 'intoxication' type Abnormal movements Hypertonia	Acidosis 0 DNPH +++ Acetest 0/±	NH_3 N or ↑ ± Lactate N Blood count N Glucose N Calcium N	**MSUD** (specific odour)	Amino acid chromatography (plasma, urine) Blood spot for tandem MS-MS
II	**Neurological deterioration**, 'intoxication' type Dehydration	Acidosis ++ Acetest ++ DNPH 0/±	NH_3 ↑ +/++ Lactate N or ↑ ± Blood count: leukopenia, thrombopenia	**Organic acidurias (MMA, PA, IVA, MCD)** **Ketolysis defects**	OAC by GLCMS (urine, plasma) Carnitine (plasma) Carnitine esters by tandem MS (urine, plasma) Blood spot for tandem MS-MS
	Neurological deterioration, 'energy deficiency' type, with liver or cardiac symptoms	Acidosis ++/± Acetest 0 DNPH 0	Glucose N or ↑ + Calcium N or ↓ + NH_3 ↑ ±/++ Lactate ↑ ±/++ Blood count N Glucose ↓ +/++ Calcium N or ↓ + Hypoketotic hypoglycaemia	**Fatty acid oxidation and ketogenesis defects** (GA II, CPT II, CAT, VLCAD, MCK-AT, HMG-COA lyase)	As above Loading test Fasting test Fatty acid oxidation studies on lymphocytes or fibroblasts
III	**Neurological deterioration**, 'energy deficiency' type Polypnoea Hypotonia Lactic acidosis, sometimes well tolerated	Acidosis +++/+ Acetest ++/0 Lactate +++/+ Lactic acidosis	NH_3 N or ↑ ± Blood count: anaemia or N Glucose N or ↓ ± Calcium N	Congenital lactic acidoses (pyruvate carrier, PC, **PDH**, Krebs cycle enzymes, (respiratory chain) **MCD**	Plasma redox states ratios (L:P, 3OHB:AA) OAC (urine), AAC (plasma) Polarographic studies Enzyme assays (muscle, lymphocytes, fibroblasts) molecular analysis
IV a	**Neurological deterioration**, 'intoxication' type Moderate hepatocellular disturbances Hypotonia, seizures, coma	Acidosis 0 (alkalosis) Acetest 0/+ DNPH 0	NH_3 ↑ +/+++ Lactate N or ↑ + Blood count N	**Urea cycle defects** **HHH syndrome** **Fatty acid oxidation defects** (GA II, CPT II, VLCAD, LCHAD, CAT), **PA, MMA, IVA**	AAC, OAC (plasma, urine) Orotic acid (urine) Liver or intestine enzyme studies (CPS, OTC) replaced now by molecular analysis

Table 1.6. *Continued*

Types	Clinical type	Acidosis/ ketosis	Other signs	Most usual diagnosis (disorder/enzyme deficiency)	Elective methods of investigation
IV 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 **3PGD** **B6-dependency** **PNPO, neurotransmitter defects** Peroxisomal defects Trifunctional enzyme Respiratory chain CDG Cholesterol biosynthesis	AAC (plasma, CSF) OAC OAC, neurotransmitters (plasma, urine, CSF) VLCFA, phytanic acid in plasma Acylcarnitine profile, OAC Lactate (plasma) Glycosylated transferrin (plasma) Sterols (plasma)
V a	**Recurrent hypoglycaemia with hepatomegaly**	Acidosis ++/0 Acetest +/0	Lactate ↑ +/++ NH3 ↑ +/0 Intractable hypoglycaemia	**Glycogenosis type I** (acetest -) **Glycogenosis type III** (acetest +) **Fructose 1,6-biphosphatase** **FAO defects** **HI**	Fasting test, loading test DNA analyses, enzyme studies (liver, lymphocytes, fibroblasts) Organic acids, acylcarnitine Insulin plasma levels
V b	Hepatomegaly Jaundice Liver failure Hepatocellular necrosis	Acidosis +/0 Acetest +/0	NH_3 N or ↑ + Lactate/+/++ Glucose N or ↓ ++	**HFI, galactosaemia** **Tyrosinaemia type I** OXPHOS defects Mitochondrial DNA depletion TALDO Mevalonic aciduria **HHH**	DNA analyses, enzyme studies Organic acids (succinyl acetone) Organic acids, enzyme/DNA analyses Polyols (tandem MS) Organic acids, enzyme/DNA
V c	**Hepatomegaly** **Cholestatic jaundice** ± failure to thrive ± chronic diarrhoea ± osteoporosis ± rickets	Acidosis 0 Ketosis 0	NH_3 N Lactate N Glucose N	Alpha-1-antitrypsin Inborn errors of **bile acid metabolism** Peroxisomal defects CDG I and II Niemann-Pick type C **LCHAD** Mevalonic aciduria Cholesterol metabolism **Cerebrotendinous xanthomatosis** Citrin deficiency	Protein electrophoresis Bile acids (plasma, urine, bile by tandem MS) VLCFA, phytanic and pipecolic Glycosylated transferrin Fibroblasts studies OAC, acylcarnitine profile OAC Plasma sterols Plasma sterols AAC (citrulline can be normal) and mutation analysis
V d	**Hepatosplenomegaly** **'Storage' signs** (coarse facies, ascites, hydrops fetalis, macroglossia, bone changes, cherry red spot, vacuolated lymphocytes) ± failure to thrive ± chronic diarrhoea ± haemolytic anaemia	Acidosis 0 Acetest 0 Ketosis 0 DNPH 0	NH_3 N Lactate N or ↑ Glucose N Hepatic signs ±/++	**Erythropoietic porphyria** GM1 gangliosidosis Sialidosis type II I-Cell disease Niemann-Pick type A/C MPS VII Galactosialidosis Glycosylated transferrin mevalonic aciduria OAC Taldo polyols	Porphyrins Oligosaccharides, sialic acid Mucopolysaccharides Enzyme studies (lymphocytes, fibroblast), Fillipin staining and mutation analysis - - Glycosylated transferrin OAC Polyols (tandem MS)

Normal values *(N)*: ammonia *(NH_3):* <80 µM; glucose: 3.5-5.5 mM; lactate *(L)* <1.5 mM. *L*, Lactate; *P*, pyruvate; *3OHB*, 3-hydroxybutyrate; *AA*, acetoacetate; ±, slight; +, moderate; ++, marked; +++, significant/massive; ↑ elevated; ↓ decreased; 0, absent (acidosis) or negative (acetest, DNPH); *ISSD*, infantile sialic acid storage disease; *MCKAT*, medium-chain 3-ketoacylCoA A thiolase; *XO*, xanthine oxidase

(◘ Table 1.6). The experienced clinician will, of course, have to interpret the metabolic data carefully, particularly in relation to time of collection and ongoing treatment. At the same time it is important to collect all the biological data listed in ◘ Table 1.5. Some very significant features (such as metabolic acidosis and, especially, ketosis) can be moderate and transient, largely depending on the symptomatic therapy. Conversely, at an advanced stage, many nonspecific abnormalities (such as respiratory acidosis, severe hyperlactataemia, secondary hyperammonaemia) can disturb the original metabolic profile. This applies particularly to IEM with a rapid fatal course, such as urea cycle disorders, in which the initial characteristic presentation of hyperammonaemia with respiratory alkalosis shifts rapidly to a rather nonspecific picture of acidosis and hyperlactataemia.

In our experience, types I and II (**MSUD, organic acidurias**), type IVa (**urea cycle defects, fatty acid oxidation disorders**) and the most common diseases in type IVb, nonketotic hyperglycinaemia and respiratory chain disorders, account for more than 80% of newborn infants with inborn errors of intermediary metabolism.

1.4 Metabolic Emergencies from Late Infancy to Adolescence

1.4.1 Clinical Presentations

Consider the possibility of an IEM in a child or adult with an acute unexplained, recurrent or refractory illness at any age. Indeed, in about 50% of patients with inborn errors of intermediary metabolism, 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 in either 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 1.7)

Acute encephalopathy is a common problem in patients (children and adults) with IEM. All types of coma can be indicative of an IEM, including those presenting with focal neurological signs. An IEM cannot be ruled out a priori by the age at onset, the accompanying clinical signs (hepatic, gastrointestinal, neurological, psychiatric, etc.), the mode of evolution (improvement, sequelae, death) or the routine laboratory data. 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 hyperammonaemia, coma with predominant hypoglycaemia and combinations of these three major abnormalities. A rather confusing finding in some organic acidurias and ketolytic defects is ketoacidosis with hyperglycaemia and glycosuria that mimics diabetic coma. The diagnostic approach to these metabolic derangements is developed below (► Chapter 4.2).

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 **organic acidaemias** and **urea cycle defects** present with focal neurological signs or cerebral oedema. These patients can be mistakenly diagnosed as having a cerebrovascular accident or cerebral tumour. In these disorders, stopping the protein intake, delivering a high-rate glucose infusion and giving 'cleansing drugs' (carnitine, sodium benzoate, etc.) can be life saving. Another treatable condition is **biotin-responsive basal ganglia disease**, which presents in childhood with a subacute encephalopathic picture of undefined origin, including confusion, vomiting and a vague history of febrile illness. Arterial tortuosity syndrome (GLUT10 deficiency) characterised by generalised tortuosity and elongation of all major arteries may result in acute infarction due to ischaemic stroke or an increased risk of thromboses.

All severe forms of **homocystinuria** (total homocysteine >100 μmol/l) can cause an acute cerebrovascular accident from late childhood to adulthood. These include **cystathionine-β-synthase deficiency** (usually B_6 responsive in the late-onset presentations), the severe **MTHFR** defects (betaine and folate responsive) and **CblC, CblD** defects (hydroxocobalamin responsive). 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 haemorrhage has also been observed in IVA, PA and MMA.

EPEMA syndrome generally starts early in infancy and is characterised by the association of progressive

Table 1.7. 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 CO_3H <10 mmol pCO_2 <25 mmHg	Ketosis + (acetest ++)	Respiratory chain defects **MCD**, PC **MMA**, **PA**, **IVA**, **GA I**, **MSUD** **Ketolysis defects** **Gluconeogenesis defects**	Diabetes Intoxication Encephalitis
		Ketosis -	**PDH**, **ketogenesis defects** **FAO**, **FDP**, EPEMA	
	Hyperammonaemia: NH_3 >100 umol/l Gaseous alkalosis pH >7.45 pCO_2 <25 mmHg Hypoglycaemia (<2 mmol/l)	Normal glucose	**Urea cycle defects*** **HHH** **LPI**	Reye syndrome Encephalitis Intoxication
		Hypoglycaemia	**FAO** (**MCAD***) **HMG-CoA lyase**	Drugs and toxin Ketotic hypoglycaemia Adrenal insufficiency GH deficiency Hypopituitarism
		Acidosis +	Gluconeogenesis defects **MSUD** **HMGCoA lyase** **FAO**	
	Hyperlactataemia: (>4 mmol/l)	Normal glucose	PC, **MCD**, Krebs cycle Respiratory chain* defects **PDH***(without ketosis) EPEMA syndrome	
		Hypoglycaemia	**Gluconeogenesis defects** (variable ketosis) **FAO** (moderate hyperlactataemia, no ketosis)	
Neurological coma (with focal signs, seizures, or intracranial hypertension)	Biological signs are very variable, can be absent or moderate; 'Metabolic coma'	Cerebral oedema Hemiplegia (hemianopsia) Extrapyramidal signs	**MSUD**, **OTC** **MSUD**, **OTC**, **MMA**, **PA**, PGK **MMA**, **GA I**, **Wilson disease*** **Homocystinuria***	Cerebral tumour Migraine Encephalitis
		Caudate nucleus and putamen necrosis	**BBGD**	
		Stroke-like	**UCD**, **MMA**, **PA**, **IVA** Respiratory chain defects (MELAS*) **Homocystinurias*** CDG **Thiamine-responsive megaloblastic anaemia** **Fabry disease*** (rarely presenting symptom) Acid maltase* deficiency (rare)	Moya-moya syndrome Vascular hemiplegia Cerebral thrombophlebitis Cerebral tumour
	Abnormal coagulation, haemolytic anaemia	Thromboembolic accidents	AT III, protein C, S **Homocystinurias*** Sickle cell anaemia CDG, PGK, GLUT 10	
Hepatic coma (hepatomegaly, cytolysis or liver failure) Reye syndrome	Normal bilirubin Slight elevation of transaminases	Steatosis and fibrosis	**FAO**, **UCD**	Reye syndrome Hepatitis Intoxication
	Hyperlactataemia	Liver failure	Respiratory chain defects	
	Haemolytic jaundice	Cirrhosis Chronic hepatic dysfunction	**Wilson disease***	
	Hypoglycaemia	Exsudative enteropathy	Hepatic fibrosis with enteropathy (**CDG Ib**)	

AT III, Antithrombin III; *BBGD*, biotin-responsive basal ganglia disease; *EPEMA*, encephalopathy, petechiae, ethylmalonic aciduria syndrome; *GH*, growth hormone; *MELAS*, mitochondrial encephalopathy with lactic acidosis stroke-like episodes; *PGK*, phosphoglycerate kinase. Treatable disorders are shown in **boldface type**. *Reported in adult cases as presenting or preponderant symptom

encephalopathy with mental retardation, pyramidal signs, and bilateral lesions in the striatum, resembling Leigh syndrome, relapsing petechiae, orthostatic acrocyanosis and recurrent attacks of metabolic decompensation with lactic acidosis without ketosis.

Two patients with 3-hydroxyisobutyric aciduria presenting with recurrent episodes of vomiting and ketoacidotic coma have been described [20]. Patients with mitochondrial DNA mutations have presented with cyclical vomiting associated with intermittent lactic acidosis [21]. GA type I frequently presents with an encephalopathic episode mimicking encephalitis, in association with an intercurrent gastrointestinal or viral infection. MELAS syndrome is another important diagnostic consideration in such late-onset and recurrent comas. 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. In rare cases, **Wilson disease** can 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). Abnormal neuroimaging studies including stroke-like lesions have been described in HHH syndrome. Aicardi-Goutières syndrome with mutations in the *SAMDH* gene may present with strokes and inflammatory syndrome with chilblains [22, 23] (► Section 1.6).

In summary, all these disorders should be considered in the differential diagnosis of 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, hyperlactataemia, or hyperammonaemia is present. In fact, these apparently initial acute manifestations are frequently preceded by other premonitory symptoms, which may be unrecognised or misinterpreted. Such symptoms include acute ataxia, persistent anorexia, chronic vomiting, failure to thrive, hypotonia, and progressive developmental delay, all symptoms that are often observed in **urea cycle disorders**, respiratory chain defects and **organic acidurias**.

Certain features are characteristic of particular disorders. For example, macrocephaly is a frequent finding in **glutaric aciduria type I**; unexplained episodes of dehydration may occur in **organic acidurias**; and hepatomegaly at the time of coma is an important although inconsistent finding in **fructose 1,6-bisphosphatase deficiency**. Severe haematological manifestations and recurrent infections are common in **IVA**, **PA** and **MMA**. Macrocytic anaemia may be an important clue indicating a **cobalamin** or **folate disorder**.

When coma is associated with hepatic dysfunction, Reye syndrome secondary to disorders of **fatty acid oxidation** or the **urea cycle** should be considered. In adults both conditions may mimic an alcoholic hepatic encephalopathy. Hepatic coma with liver failure and hyperlactataemia can be the presenting sign of respiratory chain disorders. Finally, hepatic coma with cirrhosis, chronic hepatic dysfunction, haemolytic jaundice and various neurological signs (psychiatric, extrapyramidal) is a classic, but underdiagnosed, manifestation of **Wilson disease** and of the recently described **hepatic cirrhosis, dystonia, hypermanganesaemia syndrome** (► Section 1.5.1, ◘ Table 1.22, Chapter 38).

▪ Recurrent Attacks of Ataxia

Intermittent acute ataxia and disturbed behaviour can be the presenting signs of late-onset intermittent **MSUD** and **organic acidurias**, where they are associated with ketoacidosis and sometimes with hyperglycaemia, which can mimic diabetic ketoacidosis. Late-onset **ornithine transcarbamoylase (OTC) deficiency** and **ASS deficiency** can present with recurrent attacks of ataxia. Acute ataxia associated with peripheral neuropathy is a frequent presenting sign of **pyruvate dehydrogenase** (PDH) **deficiency**; moderate hyperlactataemia with a normal L/P ratio supports this diagnosis. Hartnup disease is a classic but very rare cause of acute recurrent ataxia. Ataxia can also be a manifestation of **GLUT1 deficiency syndrome**.

▪ Acute Psychiatric Symptoms (◘ Table 1.8)

Late-onset forms of congenital hyperammonaemia, mainly partial **OTC** deficiency, can present late in childhood or in adolescence with psychiatric symptoms. Because hyperammonaemia and liver dysfunction can be mild even at the time of acute attacks, these intermittent late-onset forms of **urea cycle disorders** can easily be misdiagnosed as hysteria, schizophrenia or alcohol or drug intoxication. **Acute intermittent porphyria** and **hereditary coproporphyria** present classically with recurrent attacks of vomiting, abdominal pain, neuropathy and psychiatric symptoms. Finally, patients with **homocysteine remethylation defects** may present with schizophrenia-like, 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 also occur in the autosomal dominant disorder neuroferritinopathy [24, 25].

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)	Differential diagnosis
Psychiatric symptoms (hallucinations, delirium, dizziness, schizophrenic-like behaviour, agitation)	Hyperammonaemia (sometimes moderate) AAC, orotic acid	Mild 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**	Hysteria
	Homocystinuria (total homocysteine >100 µM)	Stroke, seizures Myelopathy	**Methylene tetrahydrofolate reductase**	Schizophrenia
	AAC (neutral AA in urines)	Skin rashes, pellagra	Hartnup disease	
	Low serum ferritin	Dystonia, parkinsonism, pallidal necrosis	Neuroferritinopathy Hallervorden-Spatz	Wilson
	Foam cells in bone marrow	Vertical ophthalmoplegia	Niemann-Pick type C	
	None	Epilepsy, retinitis pigmentosa	Ceroid lipofuscinosis	

Treatable disorders are shown in **boldface type**

- **Dehydration** (Table 1.9)

In paediatrics, dehydration is a common consequence of diarrhoea caused by a variety of enteral or parenteral acute infections and by rare genetic intestinal disorders (glucose galactose malabsorption, lactase**,** congenital chloride diarrhoea, sucrase isomaltase, acrodermatitis enteropathica) due to gastrointestinal losses. Some IEM can present as recurrent attacks of dehydration secondary to polyuria, hyperventilation or excessive sweating. The main accompanying findings (salt wasting, ketoacidosis, failure to thrive, Fanconi syndrome) can be used to classify dehydration attributable to IEM. Carbonic anhydrase XII deficiency has recently been described in a consanguineous Israeli Bedouin kindred with autosomal-recessive hyperchlohidrosis whose only symptoms were visible salt precipitates after sweating, a preponderance of hyponatraemic dehydration, poor feeding and slow weight gain in infancy [26].

- **Reye Syndrome, Sudden Unexpected Death in Infancy (SUDI) and Near-miss**

A large number of IEM have been described that produce episodes fitting the criteria originally used to define Reye syndrome and were misdiagnosed in the past because of inadequate investigations for IEM. It is important to collect blood and urine specimens for metabolic investigations at an appropriate time in relation to the illness, since most disorders presenting with a Reye-like syndrome affecting the mitochondrial pathway, **urea cycle** and **FAO disorders** and **OA** may produce only intermittent abnormalities. In addition, in contrast to the usual belief, a normal or nonspecific urinary organic acid and acylcarnitine pattern, even at the time of an acute attack, does not exclude an inherited FAO disorder.

True SUDI caused by an IEM is a rare event despite the large number of publications on the topic and despite the fact that at least 31 metabolic defects (including also some carbohydrate disorders and respiratory chain defects) are possible causes. This assertion is not true in the 1st week of life, in which unexpected death (SUDI) or near-miss is a priori due to an **FAO disorder** until proven otherwise**.**

- **Exercise Intolerance and Recurrent Myoglobinuria**

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. The most frequent and typical disorder in this group is McArdle disease due to myophosphorylase deficiency.

In the FAO disorders, attacks of myoglobinuria occur typically after mild to moderate prolonged exercise

Table 1.9. Attacks of dehydration in the absence of diarrhoea

Leading symptoms	Other signs	Age at onset	Diagnosis (disorder/enzyme deficiency)
Ketoacidosis: 'organic acidurias'	Polyuria Polypnoea Hyperglycaemia 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' Salty sweat	Photophobia Renal Fanconi syndrome	Infancy (3-6 months)	**Cystinosis**
	Hypernatraemia, vomiting Psychomotor retardation Spasticity	Neonatal to first month	**Nephrogenic diabetes insipidus** (X-linked)
	Hyperchloraemia Metabolic acidosis Alkaline urine pH	Early infancy	**RTA type I (distal)** **RTA type II (proximal)** **RTA type IV**
	Hypoglycaemia Hepatic glycogenosis Fanconi syndrome	Early infancy	**Fanconi Bickel syndrome (GLUT II mutation)**
	Pulmonary infections Diarrhea Salty sweat	Infancy to early childhood	**Cystic fibrosis** Carbonic anhydrase XII deficiency
Salt-losing syndrome: 'adrenal dysfunctions' and tubulopathy	Severe hyponatraemia Ambiguous genitalia	End of 1st week of life	**Congenital adrenal hyperplasias**
	Unambiguous genitalia	End of 1st week of life	Hypoaldosteronism
	Ambiguous genitalia	Infancy to early childhood	Congenital adrenal hypoplasia Congenital adrenal hyperplasia, late-onset forms
	Unambiguous genitalia		Hypo- and pseudohypoaldosteronism
	Hypoketotic hypoglycaemia		**FAO (CPT I and II)**

RTA, renal tubular acidosis. Treatable disorders are shown in **boldface type**

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 enzyme** (**TF**) deficiencies, which may occur in childhood, in adolescence or later. Recurrent rhabdomyolysis has been described in a child with glutaric aciduria type I.Deficiencies of VLCAD and SCHAD may also present as myopathy.

Lipin1 gene mutations have recently been found in 60% of a series of 29 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 35).

Muscle adenylate deaminase deficiency has been suspected of causing exercise intolerance and cramps in a few patients, but the relationship between clinical symptoms and the enzyme defect is uncertain. Respiratory chain disorders can present with recurrent muscle pain and myoglobinuria from the neonatal period to adolescence. A novel mitochondrial tRNA lysine variant, m.8358A>G, which is associated with exercise intolerance, muscle weakness and fatigue, has recently been described [27]. Hyperlactataemia 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 respiratory chain disorders, muscle symptoms are often associated with cardiomyopathy or diverse neurological signs (encephalomyopathy). A case of lipoamide dehydrogenase deficiency presenting as recurrent myoglobinuria has been described in an adult. It is not yet clear whether normo- and hyperkalaemic paralysis due to

sodium channel gene mutations may present with exercise intolerance attacks.

▪ 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 Ig D.

▪ Cardiac Failure, Cardiac Dysrhythmias, Orthostatic Hypotension

Acute cardiac failure and disorders of heart rhythm can be the first signs in inborn errors of metabolism (◘ Table 1.11). The important metabolic causes of cardiomyopathy are Pompe disease, all **long-chain FAO disorders** (except CPT I deficiency), multiple acyl CoA dehydrogenase deficiency, Barth syndrome and respiratory chain disorders (◘ Table 1.12). Fatal congenital heart glycogenosis due to mutation in the *PRKAG2* gene [10] can initially mimic respiratory chain disorders, or trifunctional enzyme deficiency when generalised hypotonia is associated with cardiomyopathy. Congenital disorders of glycosylation may at times present in infancy as tamponade with pericardial effusion, multiorgan failure and characteristic cutaneous and neurological 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 recently been described.

Alternatively, heart failure may result from disturbed cardiac rhythm. In congenital hypoparathyroidism and pseudohypoparathyroidism, cardiac failure can be the consequence of severe hypocalcaemia with a prolonged QT interval on EKG. In Kearns-Sayre syndrome, atrioventricular block with syncope is a classic sign, usually associated with progressive external ophthalmoplegia and retinal degeneration (► Section 'Hyperlactacidaemias', below). In the rare disorder triosephosphate isomerase

◘ Table 1.10. Abdominal pain

With flatulence, diarrhoea, loose stools	**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** **Tyrosinaemia type I** **OTC** (late onset) MNGIE syndrome (intestinal obstruction)
With hepatomegaly and splenomegaly	Cholesterol ester storage disease Lipoprotein lipase deficiency **Lysinuric protein intolerance** Haemochromatosis Mevalonate kinase deficiency, hyperIgD syndrome
With pain in extremities	**Fabry disease** 5-Aminolevulinate dehydratase deficiency Sickle cell anaemia
With haemolytic anaemia	Coproporphyria Hereditary spherocytosis Sickle cell anaemia Nocturnal paroxysmal haemoglobinuria
With Crohn/pseudo-Crohn disease	Glycogenosis type Ib[1]
With inflammatory syndrome (fever rash, IC reactive protein)	Hyper IgD syndrome (mevalonate kinase deficiency)

Ig, immunoglobulin; MNGIE, myo-neuro-gastro-intestinal-encephalopathy. Treatable disorders are shown in **boldface type**

deficiency, which presents early in infancy as haemolytic anaemia and progressive neurological dysfunction, arrhythmia may cause sudden cardiac death. A hyperkinetic haemodynamic 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. 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 rate (premature ventricular complexes, atrioventricular block, and ventricular tachycardia) are frequent features and could be due to accumulation of long-chain acylcarnitine. In neonatal cases, the heart rate disorder may be the presenting symptom.

Orthostatic hypotension is a frequent presenting sign of dopamine β-hydroxylase deficiency. It can lead to recurrent episodes of fainting, and most patients complain of fatigue and impaired exercise tolerance. Orthostatic hypotension is also frequently observed in Menkes disease and occipital horn syndrome and in aromatic l-amino acid decarboxylase deficiency.

- **Liver Failure, Ascitis, Oedema** (◘ Table 1.13)

◘ Table 1.13 presents all disorders according to their age of onset.

◘ Table 1.11. Arrhythmias, conduction defects

Primary dysryhthmias	Adrenal dysfunction (hyperkalaemia) AMP activated protein kinase mutation (*PRKAG2*: WPW) Cardiac glycogenosis (Wolf-Parkinson-White syndrome) Triose phosphate isomerase deficiency d-2-Hydroxyglutaric aciduria (AV block) **Fatty acid oxidation disorders** (all kind of arrhythmias and conduction defects may be presenting sign) **Hypoparathyroidism** (hypocalcaemia) Kearn-Sayre syndrome (respiratory chain disorders) **Thiamine deficiency-dependent states**
With cardiac/ multiorgan failure	CDG Ia (with tamponade)
With cardiomyopathy, ◘ Table 1.12	

AV, Auriculoventricular. Treatable disorders are shown in **boldface type**

◘ Table 1.12. Cardiomyopathies

AMP activated protein kinase mutation (presenting sign)
Barth syndrome (can be the presenting sign)
CDG syndrome type Ia (with pericardial effusion, can be the presenting sign)
D-2-Hydroxyglutaric aciduria
Fabry disease
Fatty acid oxidation disorders (presenting sign)
Friedreich ataxia (presenting sign)
Glycogenosis type III and IV (can be presenting sign)
GM1 gangliosidosis
Isobutyryl-CoA dehydrogenase
Methylmalonic aciduria (Cbl C), malonic aciduria
Mucopolysaccharidosis
Muscular glycogen synthetase (presenting sign)
Pompe disease, Danon disease (presenting sign)
Propionic acidaemia (can be presenting sign)
Respiratory chain disorders (presenting sign)
Selenium deficiency
Thiamine deficiency (presenting sign: extreme emergency!)
Thiamine-responsive anaemia
3-Methylglutaconic aciduria

Treatable disorders are shown in **boldface type**

◘ Table 1.13. Liver failure (ascites, oedema) (► Section 1.3.1)

Age at onset: congenital (with hydrops fetalis)	► Section 1.3.1, ◘ Table 1.6
Age at onset: neonatal to early infancy	► Section 1.3.1, ◘ Table 1.6
Age at onset: infancy	Same defects as in neonatal period ACAD 9 α-1-Antitrypsin deficiency Congenital disorder of glycosylation (CDG) **type Ib** and Ih Cholesterol ester storage disease Cystic fibrosis Familial hepatic fibrosis with exsudative enteropathy **(CDGIb)** Fatty acid transport defect **Ketogenesis defects** Pyruvate carboxylase deficiency *S*-Adenosyl homocysteine hydrolase deficiency **Urea cycle defects** Wolman disease
Age at onset: childhood to adolescence	**Wilson disease** α-1-Antitrypsin deficiency

Treatable disorders are shown in **boldface type**

- **Pain in Extremities and Bone Crisis**

Pain in extremities can be due to bone, vascular, neurological, autonomic or complex mechanisms. Painful crisis can be observed as presenting or preponderant sign in four groups of metabolic / genetic disorders. All Bone pain is a frequent symptom in **calciferol metabolism deficiency** and hereditary hypophosphataemic rickets, where it is associaterd with bone changes (rickets).

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

Neurological pain can occur in three progressive neurological disorders. In the infantile form of Krabbe disease, bone crises are expressed by irritability and incessant crying, which may precede the characteristic psychomotor deterioration with peripheral neuropathy by a few weeks. Similar crises have been observed in defects of **biopterin synthesis** and in aromatic amino acid decarboxyase deficiency. In late infantile metachromatic leukodystrophy, crises are associated with limb hypertonia, muscle weakness and progressive neurological deterioration. In Gaucher disease type III, painful crises can precede, accompany or follow a large variety of neurological symptoms (myoclonic jerks, ophthalmoplegia, spasticity, abnormal movements, etc.); they appear in late childhood or adolescence and are associated with splenomegaly.

Finally, painful crisis may occur as an isolated sign. In **erythropoietic porphyria** pain and itching, sometimes without obvious erythema and swelling, can occur within minutes of sun exposure, but in most cases there is a diffuse oedema of sun-exposed areas, which may resemble angioneurotic oedema. 'Bone crisis' is frequently the presenting symptom in hemizygotic **Fabry disease** and nonneuronopathic **Gaucher disease** (type I). The 'Fabry crisis' can last from minutes to several days. It consists in agonising 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 rate, 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. 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.

Many patients with Gaucher disease type I (chronic nonneuronopathic) 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. Occasionally, bone crisis precedes hepatosplenomegaly and is the presenting sign of the disease.

Autonomic features clinically characterised by loss of pain and temperature sensation in feet and hands, often accompanied by lancinating pain attacks, sweating disturbances and chronic skin ulcers are characteristic and frequent presenting symptoms of adult hereditary sensitive autonomous neuropathy type I (▶ Chapter 35).

1.4.2 Metabolic Derangements and Diagnostic Tests

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 1.5 must be collected at the same time during the acute attack and both before and after treatment.

- **Metabolic Acidosis** (◘ Fig. 1.2)

Metabolic acidosis is a very common finding in paediatrics. 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 unrecognised 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 hyperlactataemia. FAO and gluconeogenesis defects display a concomitant fasting hypoglycaemia. Although **fructose 1,6-bisphosphatase** deficiency is classically considered to give rise to ketoacidosis, some patients have had relatively low concentrations of ketone bodies during hypoglycaemia. When metabolic acidosis occurs with a normal anion gap and without hyperlactataemia or hypoglycaemia, the most frequent cause is renal tubular acidosis (RTA) type I or II. Pyroglutamic aciduria can also present early in life with permanent, isolated metabolic acidosis, which can be mistaken for RTA type II.

A number of IEM cause a metabolic acidosis with associated ketosis. The range of serum ketone body con-

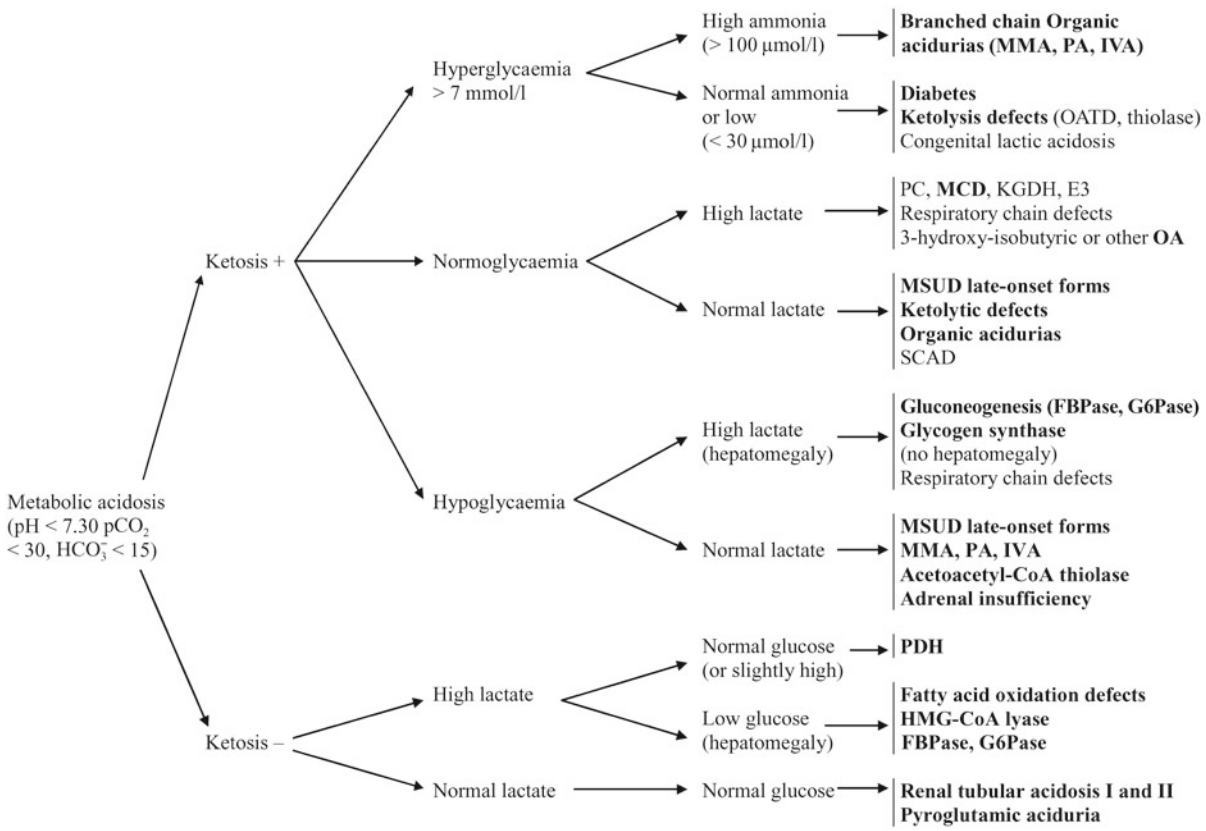

Fig. 1.2. Metabolic acidosis. *E3*, lipoamido oxido reductase; *KGDH*, alpha ketoglutarate dehydrogenase; *OATD*, oxoacid CoA transferase. Treatable disorders are shown in **boldface type**

centration varies with age and nutritional state. **Insulin-dependent diabetes**, inborn errors of **branched-chain amino acid metabolism**, congenital lactic acidoses such as **multiple carboxylase deficicency** and pyruvate carboxylase deficiency, inherited defects in enzymes of **gluconeogenesis** and of glycogen synthesis (**glycogen synthase deficiency**) and **ketolytic defects** 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 hyperglycaemia, the classic diagnosis is diabetic ketoacidosis. However, organic acidurias such as **PA, MMA or IVA** and **ketolytic defects** can also be associated with hyperglycaemia and glycosuria, mimicking diabetes. Hyperglycaemia resolves quickly within a few hours once an insulin infusion is started. 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.

In the case of hypoglycaemia, the first group of disorders to be considered is that of **gluconeogenesis** and **glycogenosis defects**. The main findings suggestive of this group are hepatomegaly and hyperlactataemia, although they are not constant. When there is no significant hepatomegaly, late-onset forms of **MSUD** and **organic acidurias** and **glycogen synthetase** (GS) deficiency should be considered. A classic differential diagnosis is **adrenal insufficiency**, which can cause a ketoacidotic attack with hypoglycaemia.

If the glucose level is normal, congenital lactic acidosis must be considered in addition to the disorders discussed above. According to this schematic approach to inherited ketoacidotic states, a simplistic diagnosis of fasting ketoacidosis or ketotic hypoglycaemia 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

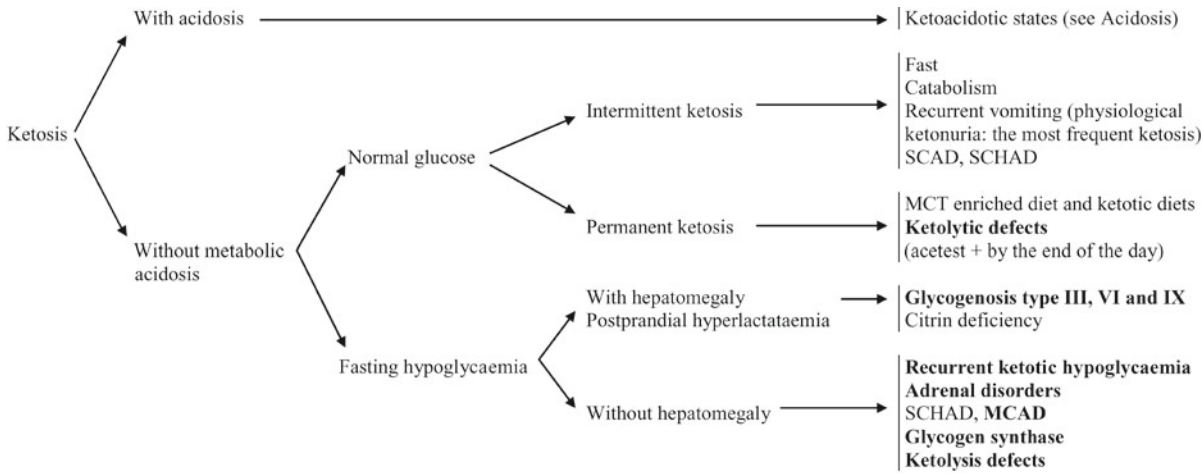

Fig. 1.3. Ketosis (see also Fig. 1.2). *MCT*, medium chain triglycerides. Treatable disorders are shown in **boldface type**

late infancy, childhood, and even adolescence. However, as a general rule, hyperketosis >6 mmol/l of total ketone bodies that produces a metabolic acidosis (serum bicarbonate <18 mmol/l) is not physiological. Ketosis which is not associated with hepatomegaly, acidosis, hyperlactataemia or hypoglycaemia 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 are ketolytic defects (**succinyl-CoA transferase** and **3-ketothiolase** deficiencies) that can present with moderate ketonuria occurring mainly in the fed state at the end of the day.

Significant fasting ketonuria without acidosis is often observed in **glycogenosis type III** in childhood (with marked hepatomegaly) and in **glycogen synthase** defect in infancy (with normal liver size). In both disorders, there is fasting ketotic hypoglycaemia and moderate postprandial hyperlactataemia and hyperglycaemia. Fasting ketonuria without acidosis is mostly observed in **ketotic hypoglycaemias** of childhood, a frequent condition which presents with recurrent attacks of vomiting with fasting ketotic hypoglycaemia and is generally triggered by episodes of catabolism. It presents most commonly between 1 and 8 years of age, is more common in children who were small for dates or those with a relatively large head circumference compared with their rather small height and weight (which suggests a gap between a low glucose hepatic production and a high glucose brain consumption). This benign condition spontaneously improves beyond about 8 years. Treatment consists of avoiding fasting >12 h and a high-calorie emergency dietary regimen when children are unable to feed normally, as can occur during intercurrent infections. Uncooked corn starch or '*crème anglaise*' (composed of milk, egg and corn starch) can also be given before bed. Ketosis without acidosis in association with fasting hypoglycaemia is also a feature of **adrenal failure**, a severe condition that requires a specific emergency treatment and which may be a presenting sign in X-ALD.

Absence of ketonuria in hypoglycaemic states, as well as in fasting and catabolism (such as with vomiting, anorexia or intercurrent infections), **is an important observation, suggesting an inherited disorder of FAO or a ketogenesis disorder**. It can also be observed in hyperinsulinaemic states at any age and in growth hormone deficiency in infancy. However, short-chain 3-hydroxy acyl-CoA dehydrogenase (SCHAD) and short-chain (SCAD) and medium-chain (**MCAD**) acyl-CoA dehydrogenase deficiencies can present as recurrent attacks of ketotic hypoglycaemia, as these enzymes are both sufficiently far down the β-oxidation pathway to be able to generate some ketones from long chain fatty acids.

- **Hyperlactataemia** (Fig. 1.4)

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 enzyme lactate dehydrogenase catalyses the lactate synthesis from pyruvate using NADH as the 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 reflect the redox state of the cells (NADH/NAD ratio).

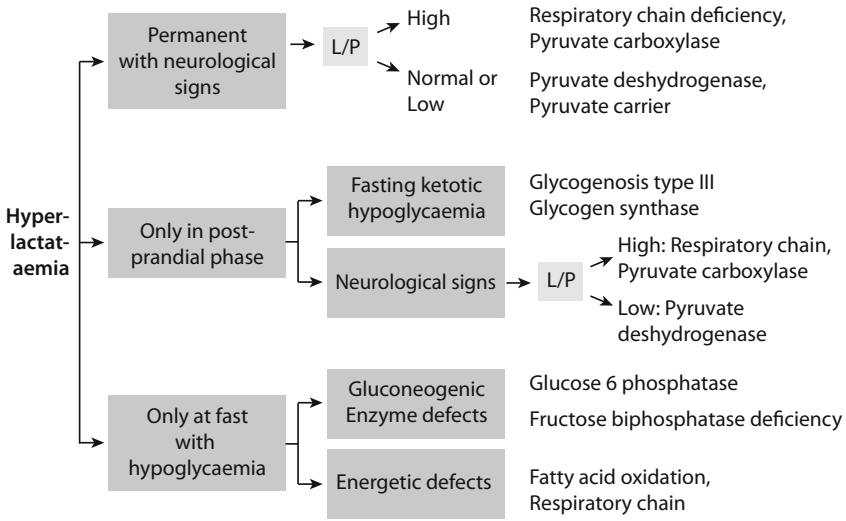

Fig. 1.4. Diagnostic approach to hyperlactacidaemia. *GSD*, Glycogenosis; *RC*, respiratory chain

Hyperlactataemia can be due to an elevation of pyruvate, the NADH/NAD ratio, H^+ (acidosis) or all of these.

Blood lactate accumulates due to elevation of the NADH/NAD ratio in circulatory collapse, in hypoxic insult and in other conditions involving failure of cellular respiration, and in all severe acidotic states. These conditions must be excluded before an inborn error of lactate-pyruvate oxidation is sought. Persistent hyperlactataemias 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 hyperlactataemias secondary to tissue hypoxia, while it is a nearly constant finding in most inborn errors of metabolism (except in **PDH deficiency**, **glycogenosis type I** and **FAO disorders**). On the other hand, the level of lactate is not discriminatory; some acquired disorders are associated with very high levels, whereas it is only moderately raised in some inborn errors of lactate-pyruvate metabolism. The nutritional state also influences the levels of lactate and pyruvate.

Once the **OAs**, **urea cycle defects** (mainly **citrullinaemia**) and **FAO defects** that can cause secondary moderate hyperlactataemia have been excluded as possible diagnoses, four types of inherited disorders remain to be considered: disorders of liver glycogen metabolism, disorders of liver gluconeogenesis, abnormalities of lactate-pyruvate oxidation (PDH, PC and Krebs cycle defects) and deficient activity in one of the components of the respiratory chain. The diagnosis of hyperlactataemias is firstly based on the clinical context. Hyperlactataemia in a context of hypoglycaemia suggests a gluconeogenesis or a glycogen metabolism defect. Hyperlactataemia in a neurological context suggests an oxidative disorder. Then the diagnosis is orientated according to two criteria, as for the diagnostic approach to hypoglycaemias:

- *Time of occurrence of hyperlactataemia relative to feeding.* In disorders of gluconeogenesis (**fructose 1,6-bisphosphatase** and **glucose-6-phosphatase deficiencies**), hyperlactataemia reaches its maximum level (up to 15 mmol/l) when the patient is fasting, acidotic and hypoglycaemic. By contrast, in **glycogenosis types III and VI** and in **glycogen synthase deficiency**, hyperlactataemia is observed only in the postprandial period in patients on a carbohydrate-rich diet. Here, hyperlactataemia never exceeds 6 mmol/l and therefore there is no acidosis (bicarbonate >18 mmol/l). In pyruvate carboxylase deficiency severe hyperlactataemia (>7 mmol/l) is present in both the fed and the fasted state, but tends to decrease with a short fast. In disorders of **PDH**, alpha ketoglutarate dehydrogenase and respiratory chain function, maximum lactate levels are observed in the fed state (although all hyperlactataemias exceeding 7 mmol/l appear more or less permanent). In these disorders, there is a real risk of missing a moderate (although significant) hyperlactataemia when the level is checked only before breakfast after an overnight fast (as is usual for laboratory determinations).
- *Determinations of L/P and ketone body ratios before and after meals.* These ratios indirectly reflect cytoplasmic (L/P) and mitochondrial (3OHB/AA) redox potential states. They must be measured in

carefully collected blood samples. Three abnormal hyperlactataemia / hyperpyruvicaemia profiles are nearly pathognomonic of an inborn error of lactate-pyruvate metabolism.

When hyperpyruvicaemia (>0.3 mmol/l) is associated with a normal or low L/P ratio (<12) without hyperketonaemia, PDH deficiency or pyruvate transporter defect [28] is highly probable, regardless of the lactate level.

When the L/P ratio is very high (>30) and is associated with postprandial hyperketonaemia and with a normal or low 3OHB/AA ratio (<1.5), a diagnosis of pyruvate carboxylase (PC) deficiency (isolated or secondary to **biotinidase** or **holocarboxylase synthetase deficiency**) or alpha ketoglutarate dehydrogenase deficiency is virtually certain. In PC deficiency, there is a very characteristic amino acid profile with hyperammonaemia, high citrulline and low glutamine.

When both L/P and 3OHB/AA ratios are elevated and associated with a significant postprandial hyperketonaemia, respiratory chain disorders should be suspected.

All other situations, especially when the L/P ratio is high without hyperketonaemia, are compatible with respiratory chain disorders, but all acquired anoxic conditions should also be ruled out (► above).

■ Hypoglycaemia (◘ Fig. 1.5)

The clinical approach to hypoglycaemia is based on four major clinical criteria: liver size, the characteristic timing of hypoglycaemia (unpredictable, only postprandial or only after fasting), association with lactic acidosis and association with hyperketosis or hypoketosis. Other clinical findings of interest are hepatic failure, vascular hypotension, dehydration, short stature, neonatal body size (head circumference, weight and length) and evidence of encephalopathy, myopathy or cardiomyopathy. Based on the liver size, hypoglycaemias can be classified into two major groups:

- *Hypoglycaemia with permanent hepatomegaly*. Hypoglycaemia associated with permanent hepatomegaly is usually due to an IEM. When hepatomegaly is the most prominent feature without liver insufficiency, gluconeogenesis defects (**glucose-6-phosphatase deficiency, fructose bisphosphatase deficiency** (**FBPase**) and **glycogenosis type III** are the most likely diagnoses. Disorders presenting with hepatic fibrosis and cirrhosis, such as hereditary **tyrosinaemia type I**, can also give rise to hypoglycaemia. The late-onset form of **hereditary fructose intolerance** rarely presents with isolated postprandial fructose-induced hypoglycaemic attacks. *S*-Adenosyl homocysteine hydrolase deficiency presents with fasting hypoglycaemia and hepatocellular insufficiency, often triggered by high protein or methionine ingestion, and is associated with hepatic fibrosis, mental retardation and marked hypermethioninaemia. Respiratory chain disorders can present with hepatic failure, hypoglycaemia and fasting lactic acidosis, which can mimic FBPase deficiency. **CDG** types Ia and **Ib** (**phosphomannose isomerase** deficiency with hepatic fibrosis and exsudative enteropathy) can cause hypoglycaemia early in infancy.
- *Hypoglycaemia without permanent hepatomegaly*. It is important to determine the timing of hypoglycaemia and to look for metabolic acidosis and ketosis when the patient is hypoglycaemic. Unpredictable postprandial hypoglycaemic attacks occurring after a very short fast (2-6 h) and without ketosis are mostly due to **hyperinsulinism** (**congenital or Munchausen by proxy**) at any age, or to **growth hormone deficiency** or related disorders in early infancy.

Most episodes of hypoglycaemia that are due to IEM and 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 hypoglycaemia without ketosis strongly suggests **FAO disorders** (without severe acidosis), **HMG-CoA lyase deficiency** or **HMG-CoA synthetase deficiency** (with acidosis). When ketoacidosis is present at the same time as hypoglycaemia, **OAs**, **ketolytic defects**, **late-onset MSUD** and glycerol kinase deficiencies should be considered, but hypoglycaemia is very rarely the presenting metabolic abnormality in these disorders. **Adrenal insufficiencies** should be always considered in the differential diagnosis, especially when vascular hypotension, dehydration, and hyponatraemia are present. Fasting hypoglycaemia with ketosis and occurring mainly in the morning and in the absence of metabolic acidosis suggests recurrent functional ketotic hypoglycaemia, 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 on occasions present as recurrent attacks of ketotic hypoglycaemia, as can **glycogen synthase deficiency**. ◘ Fig. 1.5 summarises the simplified diagnostic approach to hypoglycaemia.

■ Hyperammonaemia

For a discussion of this topic the reader is referred to ► Chapter 20, 'Disorders of the Urea Cycle and Related Enzymes.'

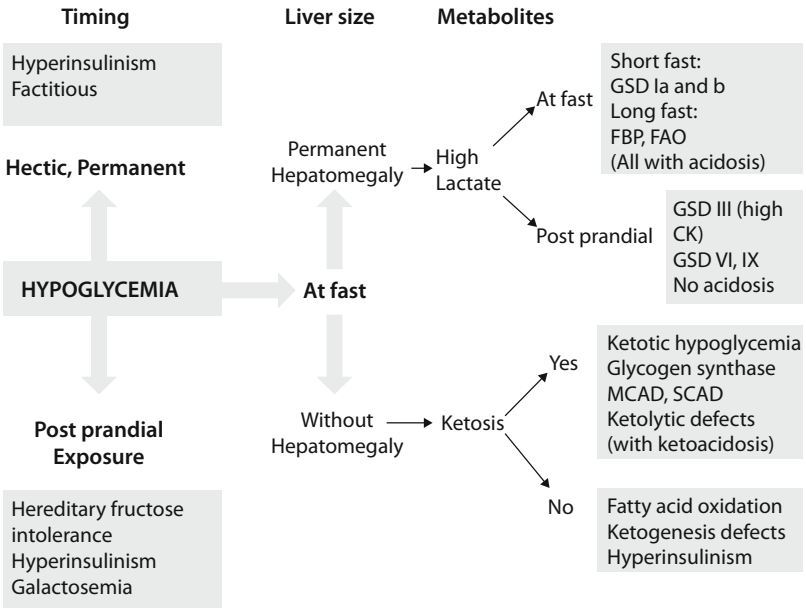

Fig. 1.5. Diagnostic approach to IEM with hypoglycaemia in paediatrics, based first on the timing of hypoglycaemia, the size of the liver and the metabolic profile

■ **Hyperuricaemia and Hypouricaemia** (► Chapter 36)

Normal uricaemia is about 5 mg/dl in men, 4 mg/dl in women and 3-4 mg/dl in children.In children a plasma uric acid level >6 mg/dl 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. Indeed, UA filtered by glomeruli is reabsorbed in the proximal tubule; urinary UA comes from distal secretion which is competitive with organic acids (lactic, methylmalonic, propionic, etc.). Several tubular UA transporters have been already described, *SLC22A12* (type I) *SLC2A9* (type II) and GLUT 9 acting probably in a multimolecular complex 'transportsome' allowing cooperation between multiple transporters [29, 30] (► Section 1.6.15).

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: hyperlactacidaemias, glycogenosis type I, OAs such as MMA (in which gout crisis and hyperuricaemic nephropathy can be a presenting sign), muscular glycogenoses and fatty acid oxidation defects in acute crisis and during treatment with dichloroacetate and after a fructose load. Hyperuricaemia is a preponderant sign in the recently described HUPRA syndrome [31].

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 urines) purine nucleoside phosphorylase and PRPP-synthetase deficiencies (with low UA excretion), but is more commonly due to decreased renal tubular UA reabsorbtion. Renal hypouricaemia due to renal UA transporter defects is characterised by blood uric acid <20mg/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.

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 gastrointestinal symptoms (such as failure to thrive, anorexia, vomiting; ► Section 1.6.5) or muscular (muscle weakness, hypotonia, poor muscle bulk; ► Section 1.6.9) can precede or accompany subtle neurological signs.

Neurological symptoms are very frequent in inborn errors and encompass progressive psychomotor retarda-

tion, seizures and a number of neurological abnormalities in both the central and peripheral system, sensorineural defects and psychiatric symptoms.

A large number of inborn errors of intermediary metabolism present with an early and nonspecific progressive developmental delay, poor feeding, hypotonia, some degree of ataxia and, frequently, 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. Many aminoacidopathies that were first described in the late 1950s and 1960s, when plasma and urine amino acid chromatography was systematically used in studying mentally retarded children, must now be questioned as definite causes of neurological disease. This is the case for histidinaemia, hyperlysinaemia, hyperprolinaemia, alpha aminoadipic aciduria, saccharopinuria, Hartnup 'disease' and the recently described acetyl aminoaciduria due to aminoacylase I deficiency [32].

A similar picture is now emerging with OAs, and it is therefore important to link clinical symptoms and metabolic disturbances. Conversely, it becomes more and more difficult to screen patients on clinical grounds when the clinical symptoms are all rather nonspecific features, such as developmental delay, microcephaly, hypotonia or convulsions. Among the new categories of inborn errors of intermediary metabolism 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 and a number of other inborn errors. These disorders rarely, if ever, cause true developmental arrest; rather, they cause progressive subacute developmental delay. Conversely, there is still an important gap between neurological descriptions and biological investigations. Many well-known heritable neurological or polymalformative syndromes have not been considered from a pathophysiological perspective and should be submitted to a comprehensive biochemical evaluation. This is illustrated for example by the story of Canavan disease, in which *N*-acetylaspartic aciduria was not found until 1988, even though the clinical phenotype had been identified in 1949 and the procedure for identifying *N*-acetylaspartate in urine was available in 1972.

1.5.1 Diagnostic Approach to Neurological and Mental Deterioration Related to Age

◘ Table 1.14–1.25 present a general approach to inborn errors of metabolism 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 neonatal period (birth to 1 month; ◘ Table 1.4, Table 1.6; ◘ Fig. 1.1) and those presenting intermittently as acute attacks of coma, lethargy, ataxia, or acute psychiatric symptoms (◘ Table 1.7, 1.8) were discussed earlier.

- **Early Infancy** (◘ Table 1.14, Table 1.15)

Three general categories can be identified.

Category 1: Disorders associated with extraneurological features (◘ Table 1.14). Visceral signs appear in lysosomal disorders. A cardiomyopathy (associated with early neurological dysfunction, failure to thrive, and hypotonia), sometimes responsible for cardiac failure, is suggestive of respiratory chain disorders, Barth syndrome, D-2-hydroxyglutaric aciduria (with atrioventricular block), or CDG (► Section 1.6.1). Abnormal hair and cutaneous signs appear in Menkes disease, Sjögren-Larsson syndrome, **biotinidase deficiency** and respiratory chain disorders. Peculiar fat pads on the buttocks and thick and sticky skin (like tallow, peau d'orange) and inverted nipples are highly suggestive of CDG. A generalised cyanosis that is unresponsive to oxygen suggests methemoglobinaemia, which is associated with severe hypertonicity in cytochrome-b5-reductase deficiency. Kernicterus and athetosis are complications of Criggler-Najjar syndrome. The EPEMA syndrome is characterised by an orthostatic acrocyanosis, relapsing petechiae, pyramidal signs, mental retardation and recurrent attacks of lactic acidosis (► Section 1.6.2). The presence of megaloblastic anaemia suggests an inborn error of **folate** and **cobalamin** (Cbl) metabolism (► Section 1.6.6). Ocular abnormalities, such as cherry red spot, optic atrophy, nystagmus, abnormal eye movements and retinitis pigmentosa, can be extremely helpful diagnostic signs (► Section 1.5.2).

Category 2: Disorders with specific or suggestive neurological signs (◘ Table 1.15). Predominant extrapyramidal symptoms are associated with inborn errors of biopterin aromatic-amino-acid metabolism, **pyridox(am)ine phosphate oxidase deficiency**, Lesch-Nyhan syndrome, cytochrome-b5-reductase deficiency, Criggler-Najjar syndrome, the early-onset form of GA type I, and **cerebral creatine deficiency**.

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** . The clinical picture in *SUCLA2* pa-

Table 1.14. Progressive neurological and mental deterioration with extraneurological symptoms (1-12 months)

Leading symptoms	Other signs	Diagnosis (disorder/enzyme deficiency)
Visceral signs	Hepatosplenomegaly Storage signs, coarse facies	Landing, I-cell disease Sialidosis type II, Niemann-Pick A Lactosyl ceramidosis
	Hepatosplenomegaly Opisthotonos, spasticity	Gaucher type II
	Hepatomegaly Retinitis pigmentosa Liver disease with sensorineural hearing loss and cataract	Peroxisomal defects CDG syndrome CCS mutations (copper chaperone for superoxide dismutase)
Hair and cutaneous symptoms	Steely brittle hair	Menkes (X-linked) Trichothiodystrophy
	Trichorrhexis nodosa	**Argininosuccinic aciduria**
	Ichthyosis, spastic paraplegia	Sjögren-Larsson syndrome **Serine deficiency syndrome**, CDG
	Alopecia, cutaneous rashes	**Biotinidase** Respiratory chain defects
	Peculiar fat pads on buttocks	CDG
	Cyanosis, hypertonicity	Cytochrome b-5 reductase
	Kernicterus, athetosis	Crigler-Najjar
	Acrocyanosis, petechiae	EPEMA syndrome
Megabloblastic anaemia	Failure to thrive, RP	**Folate and cobalamin defects** **UMP synthase deficiency**
Intracranial bleeding	Vitamin K deficiency, seizures	CDG-IIL (*COG6* mutations)
Cardiac symptoms	Cardiomyopathy Heart failure, heartbeat disorders	D2-Hydroxyglutaric acidaemia Respiratory chain defects, CDG Barth syndrome
Ocular symptoms	Cherry red spot, hydrops fetalis	Landing Galactosialidosis, sialidosis type I
	Myoclonic jerks, macrocephaly	Tay-Sachs, Sandhoff
	Optic atrophy, macrocephaly	Canavan
	Nystagmus, dystonia, stridor	Pelizaeus-Merzbacher (X-linked)
	Retinitis pigmentosa	See ► Section 1.5.1, ◘ Table 1.8
	Abnormal eye movements	Aromatic amino acid decarboxylase
	Strabismus	CDG
	Supranuclear paralysis	Gaucher, Niemann-Pick type C

RP, Retinitis pigmentosa; *UMP*, uridine monophosphate. Treatable disorders are shown in **boldface type**

tients is highly homogeneous and comprises early-onset encephalomyopathy, dystonia, deafness and Leigh-like MRI abnormalities. Macrocephaly with a startle response to sound, incessant crying and irritability are frequent early signs in GM-2 gangliosidosis, Canavan disease, Alexander leukodystrophy, infantile Krabbe disease and GA type I. Macrocephaly can also be an initial sign in 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 neurological crisis associated with progressive neurological and mental deterioration

Table 1.15. Progressive neurological and mental deterioration (1-12 months)

Leading symptoms	Other signs	Diagnosis (disorder/enzyme deficiency)
With suggestive neurological signs		
Extrapyramidal signs	Major parkinsonism	**Inborn errors of biopterin metabolism**
	Abnormal neurotransmitters	**Aromatic amino acid decarboxylase** **Tyrosine hydroxylase, PNPO**
	Choreoathetotis, self-mutilation	Lesch-Nyhan (X-linked)
	Bilateral athetosis, hypertonicity	Cytochrome b5 reductase
	Dystonia, stridor	Pelizaeus Merzbacher (X-linked)
	Kernicterus syndrome	**Criggler-Najjar**
	Acute-onset pseudoencephalitis	**Glutaric aciduria type I (GA I)**
	Low cerebral creatine	**Creatine deficiency (GAMT)**
	Spastic paraplegia, ataxia, epilepsy	**Cerebral folate deficiency**
	Leigh syndrome	PDH, complex I,SUCLA2
Painful pyramidal hypertonia	Opisthotonos	Krabbe, Gaucher III, Nieman-Pick type C
Early epilepsy infantile spasm	Spasticity Cataracts, sensorineural hearing loss, liver disease and low serum copper	NKH, SO, **untreated PKU, MSUD, OA, MCD** Menkes, CCS mutations (copper chaperone for superoxide dismutase)
Macrocephaly, startle response to sound	Cherry red spot, myoclonic jerks	Tay-Sachs, Sandhoff, Canavan, Alexander Vacuolising leukoencephalopathy
Ocular symptoms (► Section 1.5.2)	Optic atrophy, incessant crying	Krabbe (infantile)
	Dystonia, choreoathetosis	**GA I**, L-2-hydroxyglutaric aciduria
	Progressive irritability	Respiratory chain, peroxisomal defects
Recurrent attacks of neurological crisis	Failure to thrive, hyperventilation attacks	Leigh syndrome (PC, PDH, respiratory chain, MAMEL syndrome)
	Stroke-like episodes	**Urea cycle defects, MSUD, OA, GA I** CDG, respiratory chain (MELAS)
	Thromboembolic accidents	**Homocystinurias**, CDG
Without suggestive neurological signs		
Evidence of developmental arrest	Infantile spasms, hypsarrhythmia, autistic features	**Untreated PKU, biopterin defects** Peroxisomal defects, Rett syndrome
Nonspecific symptoms Apparently nonprogressive disorder	Autistic features Poor feeding, failure to thrive Hypotonia, seizures	**Hyperammonaemia** (late-onset subacute) 4-OH-Butyric, L2-OH, D2-OH-glutaric acidurias Mevalonic aciduria
	With diverse neurological findings simulating cerebral palsy	Adenylosuccinase, pyrimidine defects 3-Methylglutaconic, fumarase **Other OA, creatine deficiency** 3PGD, 3-phosphoserine phosphatase **Homocystinurias**, Salla **Neurotransmitter defects**, **cerebral folate deficiency** Angelman, **GLUT-1**

GAMT, guanidine-acetate methyltransferase; *MAMEL*, MMA, Methyl malonic aciduria. Treatable disorders are shown in **boldface type**

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, a phenotype associated with a number of IEM, some of which still remain to be identified. 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**, **OAs**, GA type I, CDG and respiratory chain disorders. Thromboembolic events can be the presenting sign of **classic homocystinuria** and CDG (▶ 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. A novel syndrome characterised by arrested psychomotor development, hypotonia and seizures with severe hypomyelination has recently been described [33] in a child with mutation in the *SLC25A12 gene* coding for the mitochondrial aspartate–glutamate carrier isoform 1, specific to neurons and muscle (▶ Section 1.5.2, ◘ Table 1.22, 1.24).

Category 3: Disorders with nonspecific developmental delay. A large number of inborn errors present with nonspecific 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 can be at least partly treatable. In this context, late-onset **subacute forms of hyperammonaemia** (usually **OTC** deficiency in girls) can present with an apparently nonspecific early encephalopathy and **inborn errors of neurotransmitter synthesis**, especially **dopa-responsive dystonia** due to cyclohydrolase deficiency, tyrosine-hydroxylase deficiency, and aromatic*l*amino acid decarboxylase deficiency, can masquerade as cerebral palsy. Recurrent attacks of seizures unresponsive to anticonvulsant drugs occurring in the 1st year of life is most often the presenting symptom of the **blood-brain barrier glucose transporter (GLUT-1)** defect, a disorder that is improved by a hyperketotic diet. The treatable **cerebral folate deficiency syndrome** (improved by folinic acid) should also be systematically screened for [34].

- **Late Infancy to Early Childhood (1-5 Years)**

In this period, diagnosis becomes easier. Five general categories can be defined (◘ Table 1.16).

Category 1: With visceral, craniovertebral, ocular, or other somatic abnormalities. These features associated with a slowing or regression of development suggest **mucopolysaccharidosis types I** and II, mucolipidosis type III, oligosaccharidosis, Austin disease, Niemann-Pick disease type C, Gaucher disease type III and lactosyl ceramidosis, all disorders that are usually easy to recognise. Mucolipidosis type IV, which causes major visual impairment by the end of the 1st 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 subtle or absent. 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.

Category 2: With progressive paraplegia and spasticity. Progressive paraplegia and spasticity are characteristic of six IEM. Metachromatic leukodystrophy and neuroaxonal dystrophy present between 12 and 24 months of age with flaccid paraparesis, hypotonia, and weakness. CSF protein content and nerve conduction velocity are disturbed in the former but normal in the latter. Schindler disease is roughly similar to neuroaxonal dystrophy, though it is often associated with myoclonic jerks. **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. A rapidly progressive flaccid paraparesis resembling subacute degeneration of the cord can be the presenting sign of inherited **Cbl-synthesis defects**. Spastic paraparesis is an almost constant finding in **HHH syndrome**. Infantile ascending hereditary spastic paralysis (IAHSP) due to Alsin mutation is characterised by a relatively selective early involvement of the corticospinal and corticobulbar pathway without signs of lower motor neuron involvement, whereas motor evoked potentials demonstrate predominant involvement of the upper motor neurons. MRI is normal in young patients. There is no biological marker. It is probably a heterogeneous disorder [35].

Category 3: Unsteady gait and uncoordinated movements (when standing, walking, sitting, reaching for objects, speaking, and swallowing) due to either ataxia, peripheral neuropathy, abnormal movements or myoclonia. 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 urinary organic-acid excretion and lactic acid metabolism are the late-onset forms of GM-1 and GM-2 gangliosidosis,

Table 1.16. Progressive neurological and mental deterioration (1-5 years)

Symptoms	Diagnosis (disorder/enzyme deficiency)
With visceral, craniovertebral, or other somatic abnormalities	
Coarse facies, skeletal changes, hirsutism, corneal opacities Major visual impairment, blindness Retinitis pigmentosa, deafness Cataract, joint laxity, hypotonia	MPS I, MPS II, MPS III, MLP III Mucolipidosis type IV (corneal clouding) Peroxisomal defects, Usher type II Pyrroline-5-carboxylase synthetase
With paraplegia, hypotonia, or spasticity due to corticospinal tract involvement or to peripheral neuropathy	
Flaccid paraparesis, pyramidal signs, hyperproteinorrhachia Flaccid paraparesis, no change in CSF, optic atrophy Progressive spastic diplegia, scissoring or 'tiptoe' gait	Metachromatic leukodystrophy (abnormal NCV) Neuro-axonal dystrophy Schindler (normal NCV) **Arginase** (high arginine, high orotic) **Cbl C** (subacute cord degeneration) **HHH** (recurrent attacks of hyperNH_3) Costeff syndrome (OPA3 mutation with 3-methylglutaconic aciduria) Infantile ascending hereditary spastic paralysis
With unsteady gait, uncoordinated movements due to cerebellar syndrome, sensory defects or myoclonia	
Without disturbances of organic acid excretion	
Ataxia, choreoathetosis, oculocephalic asynergia	Ataxia telangiectasia
Ataxia, difficulty in walking, mental/speech deterioration	GM1 (spastic quadriparesis, pseudobulbar signs) **BBGD** (caudate nucleus, putamen necrosis)
Ataxia, spinocerebellar degeneration, psychotic behaviour	GM2 (Tay-Sachs, Sandhoff) (late infantile form)
Ataxia, pyramidal signs, vision loss	Krabbe (late infantile, peripheral neuropathy)
Ataxia, muscular atrophy, peripheral neuropathy	CDG, trifunctional enzyme, peroxisomal defects
Seizures, myoclonic jerks, postictal coma, transient hemiplegia Progressive movement disorder, epilepsy	Alpers (hepatic signs, hyperlactataemia) Folate receptor defect (FOLR alpha)
With disturbances of organic and amino acid excretion (sometimes moderate or intermittent)	
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 hyperlactactaemia)
Ataxia, weakness, RP, myoclonic epilepsy	Respiratory chain defects, MERFF, methylglutaconic aciduria
Extrapyramidal signs	Creatine deficiency (GAMT)
Ataxia, peripheral neuropathy, RP	**LCHAD** (organic acids, acylcarnitine)
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)
With seizures and myoclonus, ataxia, frequent falling due to intention myoclonus or to the cerebellar ataxia	
Rapid mental regression, myoclonic jerks, blindness	INCL (early-flattening EEG, CLN1 mutations)
Akinetic myoclonic petit mal, RP, typical EEG pattern	LINCL (misdiagnosed with Lennox-Gastaut)
Rapid regression, myoclonic seizures, spasticity	Schindler (optic atrophy, severe osteoporosis)
Myoclonic epilepsy, volitional and intentional myoclonias, muscular weakness	MERFF, Niemann-Pick C, Gaucher III (ophthalmoplegia, hepatosplenomegaly)
Seizures and myoclonic jerks, uncoordinated movements	Alpers (hepatic symptoms, hyperlactataemia)
With arrest or regression of psychic and perceptual functions as presenting symptom	
Autistic behaviour, regression of high-level achievements, stereotyped movements of fingers	Rett syndrome (girls), sporadic (acquired microcephaly, secondary epilepsy)
Regression of high-level achievements, loss of speech	Sanfilippo (hirsutism, agitation)

INCL, Infantile ceroid lipofuscinosis (CLN1 mutations); *LINCL*, late infantile ceroid lipofuscinosis (CLN2 mutations); *NCV*, Nerve conduction velocity; *MERRF*, myoclonic epilepsy ragged red fibres; *MLP*, mucolipidosis; *RP*, Retinitis pigmentosa. Treatable disorders are shown in **boldface type**

late infantile Krabbe disease, ataxia telangiectasia and CDG; each presents 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 respiratory chain disorders. **Creatine deficiency** due to guanidinoacetate-methyltransferase deficiency can present in infancy, with an extrapyramidal disorder associated with epilepsy, neurological regression and failure to thrive. **GLUT I** can present with recurrent attacks of dystonia induced by exercise or permanent dystonic tremor. The newly described *FOLRI* gene mutations coding for folate receptor alpha present in late infancy to childhood with a progressive movement disorder, psychomotor decline, epilepsy and profound hypomyelination [34] (◘ Table 1.24).

Disorders with disturbances of organic- and amino-acid metabolism are numerous. **PDH** deficiency presents frequently with peripheral neuropathy, intermittent ataxia, dystonia and slight or moderate hyperlactataemia (► 'Hyperlactataemias' above). Several respiratory-chain disorders initially cause ataxia, intention tremor, dysarthria, epilepsy, myopathy and (eventually) multiorgan failure. **LCHAD deficiency**, L-2-hydroxyglutaric aciduria, 3-methylglutaconic aciduria, **MMA** and **PA** significantly disturb organic acid excretion, although sometimes only slightly and intermittently. In these disorders, the acylcarnitine profile determined (by tandem MS) from blood spots collected on dry filter paper can be very helpful in identifying characteristic abnormalities. 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.

Category 4: Predominant epilepsy and myoclonus. Predominant epilepsy and myoclonus 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). Late-onset forms of Niemann-Pick type C and Gaucher disease are easily suspected because of hepatosplenomegaly and supranuclear paralysis. Two other disorders must also be considered: myoclonic-epilepsy ragged red fibre (MERRF) syndrome and Schindler disease, which is similar to neuroaxonal dystrophy. A novel autosomal-recessive progressive myoclonic epilepsy-ataxia-neuropathy syndrome due to homozygous mutations in the *AFG3L2* gene has recently been described. AFG3L2 is a nuclear encoded mitochondrial protein that forms oligomeric m-AAA protease complexes, which play a major role in mitochondria ribosomal assembly and proteome quality control [36].

Category 5: Isolated developmental arrest or regression. 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. Although nonmetabolic, 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 behaviour, developmental regression, typical stereotyped hand movements and microcephaly.

▪ Late Childhood to Adolescence (5-15 Years)

It is important to recognise 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 neurological syndromes (◘ Table 1.17).

Category 1: With predominant extrapyramidal signs (parkinsonian syndrome, dystonia, choreoathetosis)

Category 2: With severe neurological and mental deterioration and diffuse central nervous system involvement. Category-2 patients have in common severe neurological dysfunction with bipyramidal paralysis, 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, Krabbe disease, juvenile GM-1 and GM-2 gangliosidoses or respiratory chain disorders. Peroxisomal biogenesis defects can also present in the 2nd decade of life, with peripheral neuropathy initially mimicking Charcot-Marie-Tooth type II disease, which then however evolves into a pyramidal syndrome, intellectual deterioration, dementia and, shortly thereafter, a neurovegetative state.

Category 3: With polymyoclonus and epilepsy. The juvenile form of ceroid lipofuscinosis (Spielmeyer-Vogt or Batten disease due to *CLN3* gene mutations), which presents with visual loss, retinitis, ataxia and (at an advanced stage) extrapyramidal signs, should be suspected with the onset of polymyoclonus and epilepsy. After puberty, La-

Table 1.17. Progressive neurological and mental deterioration (5-15 years) (► Chapter 2, adult)

Symptoms	Diagnosis (disorder/enzyme deficiency)
With predominant extrapyramidal signs, Parkinson syndrome, dystonia, choreoathetosis	
Torsion, dystonia, no mental retardation	Dystonia musculorum deformans
Dystonia in lower extremities, gait difficulties, normal IQ	**Segawa (GTP cyclohydrolase)** **Tyrosine hydroxylase** **Cerebral GLUT I**
Lens dislocation, marfanoid morphology	Classic homocystinuria
Generalised parkinsonian rigidity, scholastic failure	**Wilson disease**
Parkinsonism, reading/writing difficulties, alacrima, dysphagia due to achalasia	**Familial glucocorticoid deficiency** (with hypoglycaemia)
Dysarthria, dysphagia, cogwheel rigidity	**Biotin-responsive basal ganglia**
Walking difficulties, dystonic posture, mental regression	Panthotenate kinase (RP, acanthocytosis)
Orofacial dyskinesia	HARP syndrome (panthotenate kinase)
Acute psychosis, pallidal necrosis	Neuroferritinopathy
With diffuse central nervous system disorders, seizures, visual failure, dementia	
With hepatosplenomegaly	Niemann-Pick type C, Gaucher type III
Without visceral signs	Metachromatic leukodystrophy, X-ALD Peroxisomal biogenesis defects Krabbe, GM1 and GM2 Leigh syndrome, respiratory chain defects
With polymyoclonia	
Intellectual deterioration, loss of sight, RP	JNCL (Batten, CLN3 mutations)
Prominent seizures, myoclonic epilepsy, dementia	Gaucher type III (splenomegaly, osseous signs)
Cerebellar ataxia, cherry red spot	Late GM2 gangliosidosis (Sandhoff, Tay-Sachs)
Hepatomegaly, splenomegaly	Niemann-Pick type C
Myoclonic epilepsy, lactic acidosis	Respiratory chain defects (MERFF, etc.)
With predominant cerebellar ataxia	
a) Without significant mental deterioration	
Dysarthria, pes cavus, cardiomyopathy	Friedreich ataxia
Spinocerebellar degeneration	Other hereditary ataxias, peroxisomal defects
Chronic diarrhoea, low cholesterol, acanthocytosis	**Abetalipoproteinaemia**
Retinitis pigmentosa, peripheral neuropathy	Refsum disease, PHARC syndrome, peroxisomal defects, CDG
Oculocephalic asynergia, conjunctival telangiectasias	Ataxia telangiectasia
b) With deterioration and dementia	
	CTX, Lafora, GM1, GM2, Gaucher Niemann-Pick type C, Krabbe Metachromatic leukodystrophy, respiratory chain
With predominant polyneuropathy	
Acute attacks	**Porphyrias, tyrosinaemia type I**

Table 1.17. *Continued*

Symptoms	Diagnosis (disorder/enzyme deficiency)
Progressive	
With demyelination (low NCV)	Metachromatic leukodystrophy, Krabbe β-Mannosidase, Refsum, PHARC syndrome, peroxisomal biogenesis defects MNGIE syndrome
Predominantly axonal (normal NCV)	**LCHAD, trifunctional enzyme** **PDH, homocysteine remethylation defects** **CTX**, peroxisomal biogenesis defects, **α-Methyl-CoA racemase, serine deficiency** P5C synthetase, **ornithine aminotransferase** Leigh syndrome, respiratory chain defects **Abetalipoproteinaemia**, copper ATP7A
With psychiatric symptoms as the only presenting sign	
Behaviour disturbances, personality and character changes, mental regression, dementia, schizophrenia before any significant neurological or extraneurological sign	**OTC, homocystinurias** (CBS, MTHFR, cblc) Sanfilippo, metachromatic leukodystrophy, Krabbe, Niemann Pick C, X-ALD Leigh syndrome, JNCL (Batten), Hallervorden-Spatz (PK deficiency), **Wilson disease, CTX**, Huntington chorea (juvenile form) Neuroferritinopathy

CBS, Cystathionine β-synthase; *CTX*, cerebrotendinous xanthomatosis; *JNCL*, juvenile neuronal ceroid lipofuscinosis; *NCV*, Nerve conduction velocity; *PHARC,* polyneuropathy, hearing loss, ataxia, RP and cataract (► Chapter 35). Treatable disorders are shown in **boldface type**

fora disease should also be considered. Gaucher disease type III, late onset GM-2 gangliosidosis, Niemann-Pick disease type C and respiratory chain disorders can also begin with polymyoclonus as an early major sign.

Category 4: With predominant cerebellar ataxia. Friedreich ataxia and other hereditary ataxias should be considered and are recognised on clinical and genetic grounds. **Abetalipoproteinaemia** and ataxia telangiectasia are usually suspected because of the associated extraneurological signs. Peroxisomal disorders, CDG, Refsum disease and PHARC syndrome can all present similarly to a peripheral neuropathy and retinitis pigmentosa. The first three can be demonstrated by the analysis of plasma very long-chain fatty acids, glycosylated transferrin profile and plasma phytanic acid, respectively. Cerebellar ataxia in association with progressive mental deterioration, dementia, and epilepsy suggests Lafora disease, **cerebrotendinous xanthomatosis**, late-onset forms of gangliosidosis, Krabbe disease, Gaucher disease, Niemann-Pick disease type C and metachromatic leukodystrophy. Respiratory chain disorders also can present with a predominant ataxia.

Category 5: With predominant polyneuropathy. Porphyrias and **tyrosinaemia type I** can present with an acute attack of polyneuropathy mimicking Guillain-Barré 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), peroxisomal disorders (peroxin 7, other peroxisomal biogenesis defects, Refsum disease with demyelination and reduced nerve conduction velocities), defects of energy metabolism (Leigh syndrome, respiratory chain disorders, PDH deficiency, LCHAD and trifunctional enzyme deficiencies), abetalipoproteinaemia, CDG and a variant form of Menkes disease presenting in the same way as an X-linked distal hereditary motor neuropathy, with symptoms of distal muscular atrophy and weakness in older children or adults bearing missense mutations in *ATP7A*.

Category 6: With behavioural disturbances as the presenting signs. Some inborn errors of metabolism 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

behaviour and character change until hyperammonaemia and coma reveal the true situation (► 'Recurrent Attacks of Coma', above). **Homocystinuria** due to methylene-tetrahydrofolate-reductase deficiency has presented as isolated schizophrenia. Searching for these treatable disorders, including also **CTX** and **Wilson disease,** is mandatory.

- **Onset in Adulthood (>15 to >70 Years)**

For information on diagnosis of neurological and mental deterioration with adult onset the reader is referred to ► Chapter 2.

1.5.2 Diagnostic Approach to Neuromental Deterioration According to Neurophysiological and Neuroradiological Signs

Certain neurosensorial defects, such as deafness, neuro-ophthalmological disease and neurophysiological abnormalities, are important features of many metabolic syndromes. In the last decade neuro-imaging and brain NMR spectroscopy have become major tools in the diagnostic approach to the neurometabolic disorders.

- **Deafness**

The hearing loss in metabolic disorders is mostly sensorineural, symmetrical and predominantly of high frequencies, although in advanced stages all frequencies may be affected. Mitochondrial syndromic and nonsyndromic (*MTTS 1 MTRNR1*) defects are the most frequent causes of deafness caused by metabolic disease. Many genetic syndromes with deafness can mimic a metabolic disorder. Some of them are listed in ◘ Table 1.18, the very few types of metabolic deafness that can be prevented by treatment being shown in boldface type.

- **Abnormal Head circumference, Cephalhaematomas, Subdural Haematomas**

There are many untreated metabolic disorders in which microcephaly is a symptom of a nonspecific cerebral

◘ **Table 1.18.** Sensorineural deafness

Detectable in neonatal period to early infancy	Acyl-CoA oxidase deficiency Adenylate kinase 2: reticular dysgenesis Alport syndrome (mutations of *COL4A* gene) Cockayne syndrome CDG I n and ALG 11 CDG Copper chaperone CCS Encephalopathy with hyperkinurininuria Galactose-4-epimerase deficiency Pendred syndrome (mutations of *SLC26A4*) Rhizomelic chondrodysplasia punctata SUCLA 2 deficiency Wolfram syndrome Zellweger and variants (peroxysomal biogenesis defects)
Detectable in late infancy to childhood	Bartter syndrome type IV **Biotinidase deficiency** (biotin responsive) Infantile Refsum disease (pseudo Usher type 2) Mucopolysaccharidosis type I, II and IV Mannosidosis (alpha) Mucolipidosis type II (I cell disease) **Megaloblastic anaemia, diabetes and deafness (Roger syndrome) (B_1-responsive)** Mitochondrial nuclear gene mutations Mitochondrial DNA mutations (syndromic: MIDD,NARP, Wolframm syndrome) Neutral lipid storage disorder PHARC syndrome PRPP synthetase superactivity **Riboflavin transporter 2 defects** (Brown-Vialetto- van Laere syndrome)
Detectable in late childhood to adolescence	Beta mannosidosis Refsum disease (adult form) Usher syndrome type II MERFF, Kearn-Sayre syndrome, MELAS Hereditary sensory autonomic neuropathy type I

MIDD, Maternal inherited diabetes and deafness; *PRPP*, phosphoribosyl pyrophosphate. Treatable disorders are shown in **boldface type**

atrophy. Some disorders present at birth with abnormal head circumference. Only a few are treatable (specified in boldface type in ◘ Table 1.19). Subdural haematomas may occur in Menkes disease and glutaric aciduria type I and can be mistakenly diagnosed as child abuse.

▪ Neuroimaging Signs

Morphological evaluation is best undertaken by MRI. Cranial computer tomography (CT scan) is still important when looking for calcifications or in emergency situations. Proton MR spectroscopy is a tool for assessing brain metabolites, but is diagnostic in only a very few disorders. The most important neuro-radiological signs of metabolic disorders are listed in ◘ Table 1.20–1.24. Treatable disorders are indicated in boldface type. RNASET2 deficiency, one of the most recently described metabolic disorders, presents with cystic leukoencephalopathy mimicking congenital cytomegalovirus brain infection [37] (◘ Table 1.21). An increasing number of disorders presenting with hypomyelination have been recently described [38-44] (◘ Table 1.22).

▪ Neuro-ophthalmological Signs

These are summarised in ◘ Table 1.25 and Table 1.26. Retinitis pigmentosa is a frequent finding in a variety of IEM (involving lipid metabolism, peroxisome and mitochondria). A dehydrodolichyl diphosphate synthetase deficiency has recently been described in Ashkenasi Jews presenting with an autosomal recessive isolated retinitis pigmentosa [45] (◘ Table 1.25).

◘ Table 1.19. Metabolic disorders with macrocephaly or microcephaly

Macrocephaly	Microcephaly
Alexander disease	**Congenital:**
Canavan disease (acetylaspartaturia)	– Infant born to untreated PKU mother
Gangliosidosis GM2 (Sandhoff, Tay-Sachs)	– Sulfite oxidase deficiency
Glutaric aciduria type I	– **Serine synthesis defects improved by serine**
Krabbe disease (infantile form)	**Acquired:**
L-2-Hydroglutaric aciduria	– GLUT1
Respiratory chain disorders	– **Cerebral folate deficiency**
Cephalhaematomas, subdural haematomas	– Rett syndrome
– Menkes disease	– Many untreated disorders in which microcephaly is a symptom of a nonspecific cerebral atrophy
– **Glutaric aciduria type I**	

Treatable disorders are shown in **boldface type**

◘ Table 1.20. Basal ganglia/brain stem hyperintensities

Leigh syndrome	Other types of hyperintensities
Biotinidase deficiency	**Biotin-responsive basal ganglia disease**
Mutations in *SLC19A3* (biotin-responsive basal ganglia disease)	**Cerebrotendinous xanthomatosis***
Coenzyme Q10 deficiency	GM1 Gangliosidosis*
EPEMA syndrome	Semialdehyde succinate dehydrogenase deficiency
Fumarase deficiency	L2-Hydroxyglutaric aciduria
MAMEL syndrome	Methylmalonic aciduria (pallidum)
3-Methylglutaconic aciduria type 1	Mitochondrial cytopathies*
Pyruvate carboxylase deficiency	**Pyruvate dehydrogenase deficiency***
Pyruvate dehydrogenase deficiency	**Wernicke encephalopathy*** (thalami, brain stem)
Respiratory chain disorders	**Wilson disease***
SUCLA 2 deficiency	
Sulfite oxidase deficiency	

*Observed in adulthood. Treatable disorders are shown in **boldface type**

Table 1.21. Basal ganglia/brain deposits

Calcifications on CT scan	Metals
Aicardi-Goutières syndrome Biopterin metabolism defects (DHPR) Cockayne syndrome Congenital lactic acidaemias Folic acid metabolism defects GM2 Gangliosidosis Kearn-Sayre Respiratory chain disorders 3-Hydroxyisobutyric aciduria RNASET2 deficiency (cystic leukoencephalopathy mimicking congenital cytomegalovirus brain infection)	Hypoceruleoplasminaemia* (diffuse hypointensity) **Hypermanganesaemia with cirrhosis** Neuroferritinopathy* (pallidum) PKAN* (Hallervorden-Spatz, HARP syndrome: hypointensity: tiger eye) PLA2 G6 mutations **Wilson disease***

*Observed in adulthood. Treatable disorders are shown in **boldface type**

Table 1.22. Brain dysplasia and malformations

Gyration abnormalities	Corpus callosum agenesis
CEDNIK (snare protein mutation) ***O*-Glycosylation disorders:** – Muscle eye brain disease (*POMGnT)* – Walker-Warburg syndrome (*POMT1)* – Fukuyama (fukutin) – Congenital muscular dystrophy DMC1-C (fukutin-related protein) DMC1-D (protein large) Peroxisomal disorders (Zellweger and others) Glutamine synthetase deficiency Mitochondrial glutamate carrier defect	With gyration abnormalities (see left column) ACTH deficiency Aicardi Goutières syndrome (with calcifications) Complex II mitochondrial cytopathies (with leukodystrophy) Nonketotic hyperglycinaemia PDH deficiency (with basal ganglia abnormalities) 3-Hydroxyisobutyric aciduria

Table 1.23. Posterior fossa (and olivo-ponto-cerebellar) signs of metabolic disorders

Hypoplasia	Progressive atrophy	Dentate nuclei of the cerebellum hyperintensities
CDG	Mitochondrial cytopathies*	-
Mitochondrial cytopathies	GM2 Gangliosidosis*	**Cerebrotendinous xanthomatosis***
Peroxisomal disorders	**Niemann Pick C***	l2-Hydroxyglutaric aciduria
Congenital muscular dystrophies	**Cerebrotendinous xanthomatosis***	Mitochondrial encephalopathy*
Joubert syndrome	Sialidosis type 1*	Polyglucosan body disease*
	Ceroid lipofuscinosis*	Semialdehyde-succinate dehydrogenase deficiency*
	L-2-Hydroxyglutaric aciduria	**Wilson disease***
	Mevalonic aciduria	
	Neuroaxonal dystrophy (infantile)	
	Schindler disease	
	Smith-Lemli-Opitz syndrome	
	Succinyl-semialdehyde dehydrogenase deficiency	
	3-Methylglutaconic aciduria	

*Observed in adulthood. Treatable disorders are shown in **boldface type**

Table 1.24. White matter hyperintensity

With increased head circumference			
Alexander disease (anterior) Canavan disease **Glutaric aciduria type I** (bi-temporal atrophy) L-2-Hydroxyglutaric aciduria Mucopolysaccharidosis (with vacuoles) Vacuolising leukoencephalopathy Megalencephalic leukodystrophy with subcortical cysts (MLC1)			
Without increased head circumference			
Diffuse hypomyelination	**Predominantly periventricular white matter (sparing U-fibres)**	**Affecting U fibres**	**Pyramidal tracts**
Cerebral folate transport deficiency (mutations of FOLRI coding for folate receptor alpha)	Aicardi-Goutières syndrome (with calcifications) white matter	**Glutaric aciduria type I***	Adrenomyeloneuropathy*
Galactosaemia	CACH (vanishing white matter disease)	**Homocysteine remethylation defects***	**Cerebrotendinous xanthomatosis***
Fucosidosis	**Cerebrotendinous xanthomatosis***	L-2-Hydroxyglutaric aciduria	Krabbe disease*
GJA12 and GJA1 Connexins defects in Pelizaeus-Merzbacher-like and oculodentodigital syndromes (with present brain stem auditory EP)	Cockayne syndrome (with calcifications)	3-MethylglutarylCoA lyase deficiency*	Mitochondrial cytopathies*
Mitochondrial hsp60 chaperonopathy	**Homocysteine remethylation defects***	Mitochondrial cytopathy	
Pelizaeus-Merzbacher (with myelination arrest and absent brain stem auditory EP)	Glutaric aciduria type I*	Polyglucosan body disease*	
Ribose-5-phosphate isomerase* (arabitol, ribitol peaks)	Kearn-Sayre syndrome		
SLC17A5 (coding for sialin with sialic acid accumulated only in CSF)	Menkes disease		
Serine synthesis defects	Metachromatic leukodystrophy*		
HABC (Hypomyelination with atrophy of the basal ganglia and cerebellum)	Mitochondrial cytopathy		
SPTAN1 encoding β-II spectrin	MNGIE (with supratentorial cortical atrophy)		
TACH (Tremor-ataxia with central hypomyelination leukodystrophy	Peroxisomal biogenesis defects*, **PEX-7**		
4H syndrome (hypomyelination, hypogonadotropic hypogonadism, hypodontia) *SLC25A12* encoding for mitochondrial aspartate-glutamate carrier isoform 1	**PKU (untreated, reversible)***		
	Polyglucosan body disease*		
	X-ALD (posterior)		
	3-MethylglutarylCoA lyase deficiency*		

EP, evoked potential; *Observed in adulthood. Treatable disorders are shown in **boldface type**

Table 1.25. Abnormal fundoscopy findings

Cherry red spot	Retinitis pigmentosa and others	Optic atrophy (optic pallor)
Niemann-Pick types A, B Galactosialidosis (neuraminidase deficiency) Gangliosidosis GM1 (Landing) Gangliosidosis GM2 (Sandhoff, Tay-Sachs) Nephrosialidosis Sialidosis type I Cytochrome C oxidase deficiency	**Retinitis pigmentosa:** Abetalipoproteinaemia **Vitamin E malabsorption** (tocopherol carrier) CDG Ceroid lipofuscinosis, **CblC*** Dehydrodolichyl diphosphate synthase defect Panthothenate kinase deficiency* (Hallervorden-Spatz, HARP) Peroxisomal defects* PHARC syndrome **LCHAD**, trifunctional enzyme deficiency Respiratory chain disorders (Kearns Sayre, NARP, mtDNA deletions)* Mucopolysacchariddoses **Others:** **Gyrate atrophy with OAT deficiency** Aceruleoplasminaemia* Sjögren-Larsson syndrome Mucolipidosis type IV	**Biotinidase deficiency** Canavan disease (early sign) **CblC*** Ceroid lipofuscinosis (CLN3*, CLN4*) Krabbe disease (infantile) Leber due to mitochondrial DNA deletions* Leigh syndrome (all causes) Metachromatic leukodystrophy* 3-Methylglutaconic aciduria type 3 (Costeff optic atrophy syndrome) Mitochondrial cytopathies* Neuroaxonal dystrophy – Schindler (infantile) **Organic acidurias (MMA, PA)** Pelizaeus-Merzbacher (presenting sign early in infancy) Peroxisomal biogenesis defects* Polyprenol reductase defect (CDG I due to *SRD5A3* mutations) **Pyruvate dehydrogenase deficiency*** Ribose-5-phosphate isomerase* Sulfite oxidase (infantile) X-ALD* Wolfram syndrome

MMA/PA, Methylmalonic/propionic aciduria. *Observed in adulthood. Treatable disorders are shown in **boldface type**

Table 1.26. Ophthalmoplegia, ptosis, eye movements, strabismus

Neonatal to early infancy (oculogyric crises)	Infancy to childhood	Adulthood
Aromatic aminoacid decarboxylase deficiency (oculogyric crisis)	Ataxia telangiectasia (ocular contraversion, telangiectasia) Gaucher disease type III (horizontal supranuclear paralysis) Leigh syndrome (acute attacks) Niemann-Pick C (vertical supranuclear paralysis) **Pyruvate dehydrogenase deficiency (acute attacks)** Respiratory chain defects (acute attacks)	Niemann-Pick C disease Gaucher disease type III (see above) Mitochondrial cytopathies (mt DNA deletion) Glutaric aciduria type I GM2 gangliosidosis (abnormal eye movements) Nonketotic hyperglycinaemia **Pyruvate dehydrogenase deficiency (abnormal movements)** Ataxia with oculomotor apraxia (AOA1 and 2)
Ataxia telangectasia (ocular contraversion) **Biopterin synthesis defects** CDG Ia (with congenital strabismus) Cogan syndrome (ocular contraversion) **Pyridox(am)ine-5-phosphate oxidase deficiency** **Tyrosine hydroxylase deficiency**		

Treatable disorders are shown in **boldface type**

- **Neurophysiological Signs** (Table 1.27 and ► Section 1.5.1)

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 (cerebellar ataxia in the case of respiratory chain disorders). Many exceptions to this schematic view exist, however. MNGIE syndrome caused by thymidine phosphorylase deficiency is typically responsible for a demyelinating polyneuropathy. Some lipid storage disorders, such as cerebrotendinous xanthomatosis, adrenomyeloneuropathy and other peroxisomal diseases, may cause polyneuropathies that can be axonal, demyelinating or both.

Table 1.27. Polyneuropathy – electroneuromyographic findings

Acute (recurrent attacks)	Demyelinating (low nerve conduction velocity	Predominantly axonal (normal nerve conduction velocity)	Miscellaneous
Porphyrias*	AMN (adulthood)	**Abetalipoproteinaemia** (childhood)	**Fabry disease*** (presenting sign)
Tyrosinaemia type I*	Austin disease	(Affecting small sensitive fibres and the autonomic nervous system):	GM2 gangliosidosis*
PDH deficiency*	β-Mannosidosis deficiency	α-Methylacyl-CoA racemase deficiency (adolescence to adulthood)	**Cerebrotendinous xanthomatosis*** (leukodystrophy)
	Cerebrotendinous xanthomatosis* (leukodystrophy)	CDG type I (childhood)	**Porphyria***
	Farber lipogranulomatosis	GM2 gangliosidosis*	**Tangier disease***
	Homocysteine remethylation defects (MTHFR, CblC)	**LCHAD**, trifunctional enzyme deficiency (childhood to adolescence)	**Affecting anterior horn:**
	Krabbe disease (leukodystrophy)	Neuroaxonal dystrophy, Schindler disease (early childhood) (leukodystrophy)	GM2 gangliosidosis
	Metachromatic leukodystrophy (leukodystrophy)	**Ornithine amino transferase deficiency** (late complication)	Krabbe disease
	MNGIE syndrome (leukodystrophy)	**Pyruvate dehydrogenase deficiency** (childhood to adulthood)	Peroxisomal biogenesis defects (late childhood to adult)
	Refsum disease and PHARC syndrome	Polyglucosan body disease*	**Homocysteine remethylation defects (ClbC)**
	Tangier disease	P5C synthase deficiency (late childhood)	Polyglucosan body disease* (with leukodystrophy)
	X-ALD (childhood to adulthood): leukodystrophy	**Porphyria***	Panthotenate kinase deficiency (Hallervorden-Spatz syndrome) (basal ganglia)
		Pyroglutamic aciduria (late complication)	
		Respiratory chain defects (early childhood to adolescence)	
		Serine deficiency syndrome (adolescence)	
		Triose phosphate isomerase deficiency	
		Vitamin E malabsorption (tocopherol carrier defect)	
		Triose phosphate isomerase	

AMN, Adrenomyeloneuropathy; *MNGIE*, myoneurogastrointestinal encephalopathy; *P5C*, pyrolline 5 carboxylate. *Observed in adulthood. Treatable disorders are shown in **boldface type**

- **Self-mutilation and Auto-aggression**

These are mostly observed in Lesch Nyhan syndrome, untreated phenylketonuria, acute crises of tyrosinaemia type I and 3-methylglutaconic aciduria; however, such severe behavioural disturbance can occur in any condition that causes profound learning difficulties.

1.6 Specific Organ Signs and Symptoms

IEM can involve any organ systems 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 rather nonspecific (e.g. hepatomegaly, seizures, mental retardation). The most important are listed below in Table 1.28–1.40. The following diagnostic checklist is primarily based upon the author's personal experience and it is not, of course, exhaustive. It should be progressively extended by the experiences of all readers.

1.6.1 Cardiology

All pertinent information on cardiac failure, cardiac dysrhythmiass and cardiomyopathies is presented in ▶ Section 1.4.1 and Table 1.11 and Table 1.12.

1.6.2 Dermatology

The main presentations are summarised in Table 1.28 to Table 1.31. The most recently described disorders presenting with preponderantly dermatological signs are mutations in the human SC4MOL gene encoding a methyl sterol oxidase causing psoriasiform dermatitis, microcephaly and developmental delay [46] (Table 1.29) and perilipin deficiency presenting with an autosomal dominant partial lipodystrophy [47] (Table 1.30).

Table 1.28. Hair signs

Alopecia	Brittle hair	Pili torti	Trichorrhesis nodosa
1. Age at onset: neonatal period to infancy	ASA	Menkes disease	Argininase deficiency
Acrodermatitis enteropathica	Citrullinaemia	Netherton syndrome	ASA
Biotin-responsive MCD	Menkes disease	CDGIa	LPI
Calciferol metabolism defects	MPS	-	Menkes disease
Congenital erythropoietic porphyria	Pollitt's syndrome	-	Netherton syndrome
	Trichothiodystrophy		
Conradi-Hunermann syndrome		**Hypertrichosis**	
Ehlers-Danlos syndrome type IV		MPS typesI, II, III, VI, VII, VIII	
Essential fatty acid deficiency		MLP III	
Hepatoerythropoietic porphyria		GM1 gangliosidosis	
Menkes disease (X-linked) **Methylmalonic and propionic acidurias**		Mannosidosis	
Netherton syndrome		Taldo deficiency	
		Leigh syndrome due to CCO and other mitochondrial defects	
Zinc deficiency			
2. Age at onset: adulthood			
Porphyria cutanea tarda Steinert disease			

CCO, Cytochrome C oxidase; *Taldo*, transaldolase. Treatable disorders are shown in **boldface type**

Table 1.29. Hyperkeratosis, ichthyosis

Hyperkeratosis-dyskeratosis	Ichthyosis (with congenital erythrodermia)
CEDNIK (neurocutaneous syndrome: keratosis on palms and soles)	Austin disease (multiple sulfatase deficiency)
Ichthyosis (see right column)	CDG If, Im
Tyrosinaemia type II (keratosis on palms and soles)	CEDNIK (neuro-cutaneous syndrome: SNARE)
Hailey-Hailey disease (acantholysis and dyskeratosis): SPCA defect (mutation of *ATP2C1*)	Chondrodysplasia punctata
Darier-White disease or keratosis follicularis: SERCA ATPase defect due to mutation of *ATP2A2*)	Conradi-Hünermann syndrome (X-linked) Gaucher type II
Protoporphyria (seasonal keratosis on palms)	Multisystemic triglyceride storage disease Refsum disease (adult form) **Serine deficiency syndrome** Sjögren-Larsson syndrome Steroid sulfatase deficiency (X-linked) Sterol-C4-methyl oxidase-like gene defect (generalised ichthyosiform erythroderma not involving palms and soles)

SERCA, Sarco-/endoplasmatic reticulum; *SPGA*, Golgi-associated secretory pathway. Treatable disorders are shown in **boldface type**

Table 1.30. Vesiculous bullous lesions, photosensitivity

Photosensitivity and skin rashes	Vesiculo-bullous lesions	Acrocyanosis
Age at onset: neonatal period to childhood		
Biotinidase deficiency (rash) Congenital erythropoietic porphyria Erythrohepatic porphyria Erythropoietic protoporphyria Hartnup disease Mevalonic aciduria (rash with fever and arthralgia) Zinc deficiency (rash) Respiratory chain disorders (rash and mottled pigmentation of exposed areas)	**Acrodermatitis enteropathica** **Biotinidase deficiency** **Holocarboxylase synthetase deficiency** **LPI** (lupus like skin lesions) **Methylmalonic, propionic acidaemias** (isoleucine deficiency in too severe protein restriction) **Zinc deficiency**	EPEMA syndrome (orthostatic) Aicardi Goutières syndrome (chilblains) ► Section 1.6.16
Age at onset: adulthood		
Hereditary coproporphyria Porphyria variegata Porphyria cutanea tarda		

EPEMA, Ethylmalonic encephalopathy. Treatable disorders are shown in **boldface type**

Table 1.31. Nodules, xanthoma, angiokeratosis and miscellaneous

Angiokeratoma	Xanthoma	Nodules	Miscellaneous
Aspartylglucosaminuria β-Mannosidosis Fabry disease (presenting sign) Fucosidosis GM1 gangliosidosis Galactosialidosis Kawasaki disease Schindler disease (adult form)	Apo CII (eruptive) Apolipoprotein A1 deficiency (planar), **autosomal dominant Hypercholesterolaemia: autosomal recessive Hyperlipoproteinaemia type III** Hepatic lipase deficiency Lipoprotein lipase deficiency (eruptive) Sitosterolaemia (childhood) Cerebrotendinous xanthomatosis	CDG Farber disease	**Laxity (dysmorphic scarring, easy bruising)** Ehlers-Danlos syndrome (9 types) GLUT 10 deficiency Menkes disease, occipital horn syndrome P5C synthetase deficiency P5C reductase deficiency **Telangiectasias – purpuras – petechiae** Ataxia telangectasia (ocular) EPEMA (petechiae, acrocyanosis) CDG Ie and IIf GLUT 10 deficiency Prolidase deficiency **Ulceration (skin ulcers)** Prolidase deficiency HSAN type 1 **Lipodystrophy (partial)** Perilipin deficiency (with severe dyslipidaemia, and insulin-resistant diabetes)

HSAN, hereditary sensory autonomic neuropathy; *P5C*, pyrroline-5-carboxylate. Treatable disorders are shown in **boldface type**

1.6.3 Dysmorphology, Malformations, Dysplasia

For information on these topics the reader is referred to ► Section 1.2 and Table 1.1–1.3).

1.6.4 Endocrinology (Table 1.32)

IEM may be associated with endocrine dysfunction, most frequently with disturbances of carbohydrate metabolism (hypoglycaemia, diabetes). **Hyperinsulinism, glycogenosis, FAO disorders, carnitine cycle disorders**, glycosylation disorders, **hereditary fructose intolerance, tyrosinaemia**, X-ALD and OAs may be associated with hypoglycaemia. These diagnoses should be considered in cases of unexplained hypoglycaemia. Diabetes is related to iron overload, mitochondriopathy and **thiamine-sensitive diabetes**. The clinical spectrum of some forms of IEM can change from hypoglycaemia in childhood to diabetes in adulthood. 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

Table 1.32. Endocrine abnormalities

Pancreas	Thyroid/parathyroid	Adrenal/sexual glands	Growth hormone deficiency
Diabetes (and pseudodiabetes): Abnormal pro-insulin cleavage Diabetes, deafness and TRMA Kir 6.2, glucokinase defects Organic acidurias (MMA, PA, IVA, ketolytic defects) Respiratory chain defects Wolfram syndrome (DIDMOAD syndrome)	**Hyperthyroidism:** Glutaric aciduria (glutaryl-CoA oxidase deficiency ?)	**Hypogonadism – sterility:** CDG type I Galactosaemia D-Bifunctional protein (Perrault syndrome) X-linked ALD Fabry disease Cystinosis (male) Alstrom disease Haemochromatosis	Respiratory chain defects
		Polycystic ovary: GSD I and III	
Hyperinsulinism: SUR1 and KIR 6.2 defects Glucokinase overactivity GDH overactivity SCHAD deficiency Monocarboxylic transporter1 overactivity (*SLC16A1)*	**Hypothyroidism:** Allan-Herndon-Dudley syndrome (monocarboxylate transporter 8 defect) Respiratory chain defects Cystinosis Fabry disease	**Sexual ambiguity:** Congenital adrenal hyper- and hypoplasia Disorders of adrenal steroid metabolism	
		Adrenal failure: X-ALD Respiratory chain defect	
	Hypoparathyroidism: – **LCHAD deficiency** – Respiratory chain defects; Kearn-Sayre syndrome – **Trifunctional enzyme deficiency**	**Salt-losing syndrome:** **Disorders of adrenal steroid metabolism** **FAO defects (CPTII)** Respiratory chain defects (mitochondrial DNA deletions)	

DIDMOAD, Diabetes insipidus, diabetes mellitus, optic atrophy, and deafness; *GDH*, glutamodehydrogenase; *MNGIE*, myoneurogastrointestinal encephalopathy; *TRMA*, Thiamine responsive megaloblastic anaemia. Treatable disorders are shown in **boldface type**

hyperoxaluria. The Allan-Herndon-Dudley syndrome, an X-linked mental retardation syndrome that is due to mutations in the **monocarboxylate transporter 8 gene**, involves the transport of tri-iodothyronine into neurones and disturbs blood levels of thyroid hormone [48]. Long-term consequences of IEM for fertility, reproduction and bone metabolism are still poorly understood. Hypogonadism develops in almost all females with galactosaemia and is frequent in CDG, cystinosis and iron overload.

1.6.5 Gastroenterology

Gastrointestinal (GI) findings (anorexia, failure to thrive, osteoporosis, chronic vomiting) occur in a wide variety of IEM. Unfortunately, their cause often remains unrecognised, thus delaying the correct diagnosis. Persistent anorexia, feeding difficulties, chronic vomiting, failure to thrive, frequent infections, osteopenia and generalised hypotonia in association with chronic diarrhoea are the presenting features in a number of constitutional and acquired diseases in paediatrics. They are easily misdiagnosed as cow's milk protein intolerance, celiac disease, chronic ear, nose and throat infections, late-onset chronic pyloric stenosis, etc. Congenital immune deficiencies are also frequently considered, although only a few present early in infancy with this clinical picture. From a pathophysiological point of view, it is possible to define two groups of IEM presenting with chronic diarrhoea and failure to thrive:

- Disorders of the intestinal mucosa or the exocrine function of the pancreas with almost exclusively intestinal effects, for example congenital chloride diarrhoea, glucose-galactose malabsorption, lactase and sucrase-isomaltase deficiencies, abetalipoproteinaemia type II (Anderson disease), enterokinase deficiency, acrodermatitis enteropathica and selective intestinal malabsorption of folate and vitamin B_{12}, the last also causing systemic disease.
- Systemic disorders which also give rise to GI 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. This is summarised in ◘ Table 1.33.

■ **Abdominal Pain (Recurrent)**

For information on this symptom the reader is referred to the paragraph on 'Acute Symptoms' in ► Section 1.4.1 »Abdominal Pain (Recurrent Attacks)« and to ◘ Table 1.10.

■ **Acute Pancreatitis**

Disorders which can display acute pancreatitis are listed below:

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

■ **Chronic Diarrhoea, Failure to Thrive, Osteoporosis**

See ◘ Table 1.33 for information on this cluster.

■ **Hypocholesterolaemia**

Hypocholesterolaemia may be a significant finding in the following disorders:

- Abetalipoproteinaemia types I and II
- Congenital disorders of glycosylation type I
- Infantile Refsum disease
- Mevalonic aciduria
- Niemann Pick desease type C
- Peroxisomal disorders
- Smith-Lemli-Opitz syndrome
- Tangier disease (alpha lipoprotein deficiency)

■ **HELLP Syndrome**

HELLP syndrome has been described during the pregnancies of mothers carrying babies with certain disorders including:

- Carnitine palmitoyl transferase I deficiency
- LCHAD deficiency and other fatty acid oxidation disorders
- Respiratory chain defects

■ **Constipation, Intestinal Obstruction**

Constipation and intestinal obstruction may be presenting signs in the following disorders

- Mevalonic aciduria (acute crisis)
- Organic acidurias in early infantile acute crisis (IVA, PA, MMA)
- Porphyria crisis (constipation)
- Mitochondrial disease (MNGIE syndrome,: thymidine phosphorylase deficiency, tRNAleu (UUR), POLG mutations) [49].

1.6.6 Haematology

■ **Red Blood Cells Disturbances**

A large variety of IEM listed in ◘ Table 1.34 can cause anaemias. Over 95% of macrocytic anaemias are secondary to acquired deficiencies of folate or vitamin B_{12}, but many inherited disorders of vitamin B_{12} and folate metabolism present with macrocytic anaemia (with the notable exception of methylene tetrahydrofolate reductase deficiency). Haemolytic anaemias are due to deficiencies of glycolytic and pentose phosphate shuttle enzymes (some of them presenting with neurological signs), abnormal erythrocyte nucleotide metabolism, porphyrias, 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 [50]. The

◘ **Table 1.33.** Chronic diarrhoea, poor feeding, vomiting, failure to thrive

Leading symptoms	Other signs	Age at onset	Diagnosis (disorder/enzyme deficiency)
Severe watery diarrhoea	Nonacidic diarrhoea, hypochloraemic alkalosis	Congenital to infancy	**Congenital chloride diarrhoea**
Attacks of dehydration	Acidic diarrhoea, reducing substances in stools	Neonatal	**Glucose galactose malabsorption**
			Lactase deficiency
	Acidic diarrhoea, reducing substances in stools after weaning	Neonatal to infancy	**Sucrase isomaltase deficiency**
	Skin lesions, alopecia	Neonatal or post weaning	**Acrodermatitis enteropathica**
Protein-losing enteropathy	Cholangitis crisis	Infancy	**CDG Ib, Ic, Ih, IIa**
	Hypoglycaemia		

Table 1.33. *Continued*

Leading symptoms	Other signs	Age at onset	Diagnosis (disorder/enzyme deficiency)
Fat-soluble vitamins malabsorption Severe hypocholesterolaemia Osteopenia Steatorrhoea	Cholestatic jaundice	Neonatal to infancy	**Bile acid synthesis defects**
			Infantile Refsum disease
	Hepatomegaly, hypotonia, retinitis pigmentosa, deafness	Infancy	Infantile Refsum disease CDG type I
	Abdominal distension, ataxia, acanthocytosis, peripheral neuropathy, retinitis pigmentosa	Infancy	**Abetalipo I and II** (no acanthocytes, no neurological signs in type II)
	Pancreatic insufficiency, neutropenia, pancytopenia	Early in infancy	Pearson syndrome, Schwachman syndrome
Severe failure tothrive, anorexia, poor feeding, with predominant hepatosplenomegaly	Severe hypoglycaemia. Neutropenia inflammatory bowel disease	Neonatal to early infancy	**Glycogenosis type Ib** (no splenomegaly)
	Hypotonia, vacuolated lymphocytes	Neonatal	Wolman disease
	Adrenal gland calcifications		
	Recurrent infections	Infancy	**Chronic granulomatosis disease**
	Inflammatory bowel disease		(X-linked)
	Megaloblastic anaemia, neuropathy, homocystinuria, methyl malonic aciduria	1-5 years	**Intrinsic factor defect**
	Leukoneutropenia, osteopenia, hyperammonaemia, interstitial pneumonia	Infancy	**Lysinuric protein intolerance**
	Recurrent fever, inflammatory bowel syndrome, hyper-IgD	Infancy	Mevalonate kinase deficiency
Severe failure to thrive, anorexia, poor feeding, with megaloblastic anaemia	Oral lesion, neuropathy, infections, pancytopenia, homocystinuria, MMA	1-2 years	**Transcobalamin II** **Intrinsic factor deficiency**
	Stomatitis, peripheral neuropathy, infections, intracranial calcifications	Infancy	**Congenital folate malabsorption**
	Severe pancytopenia, abnormal marrow precursors, lactic acidosis	Neonatal	Pearson syndrome
Severe failure to thrive, anorexia, poor feeding, no significant hepatosplenomegaly, no megaloblastic anaemia	Severe hypoproteinaemia, putrefactive diarrhoea	Infancy	**Enterokinase deficiency**
	Diarrhoea after weaning, cutaneous lesions (periorificial), low plasma zinc	Infancy	**Acrodermatitis enteropathica**
	Ketoacidotic attacks, vomiting	Infancy	**Organic acidurias (MMA, PA)**
			Mitochondrial DNA deletions
	Vomiting, lethargy, hypotonia, hyperammonaemia	Infancy	**Urea cycle defects** (mainly **OTC)**
	Frequent infections, lymphopenia	Infancy	**Adenosine deaminase deficiency**
	Developmental delay, relapsing petechiae,orthostatic acrocyanosis	Infancy	EPEMA syndrome
	Skin laxity, pili torti, hypothermia, hernia	Infancy	Menkes disease
	Hypotonia, seizures, facial dysmorphism		

Ig, Immunoglobulin. Treatable disorders are shown in **boldface type**

Table 1.34. Red blood cells disturbances

Acanthocytosis	Anaemias: macrocytic	Anaemias : nonmacrocytic, haemolytic or due to combined mechanisms
Abetalipoproteinaemia Hallervorden-Spatz syndrome (panthothenate kinase deficiency) **Inborn errors of cobalamin metabolism (Cbl C)** Wolman disease	**Hereditary orotic aciduria** **Inborn errors of cobalamin metabolism:** – **Imerslund disease** – **Intrinsic factor deficiency** – **TC II deficiency** – **Cbl C, D, E, F, G** – **Methionine synthase deficiency** **Inborn errors of folate metabolism:** – Dihydrofolate reductase deficiency – Glutamate formimino transferase deficiency – **Congenital folate malabsorption** – Mevalonic aciduria – Pearson syndrome (due to mitochondrial DNA deletion) (dyserythropoiesis) – Respiratory chain disorders – **Thiamine-responsive megaloblastic anaemia**	Abetalipoproteinaemia Adenylate kinase deficiency Adenosine triphosphatase deficiency **Carnitine transport defect** **Congenital erythropoietic porphyria** Di-metal transporter 1 deficiency (DMT1 encoded by SLC11A2) **Erythropoietic porphyria** **Erythropoietic protoporphyria** **Galactosaemia** **Glycolytic and pentose-phosphate enzyme deficiencies** Haemochromatosis **IRIDA** (matriptase2 deficiency) encoded by *TMPRSS6* gene Lecithin cholesterol acyltransferase deficiency Mevalonic aciduria Mitochondrial tyrosyl-tRNA synthetase: MLASA syndrome with sideroblastic anaemia Pyroglutamic aciduria Pyrimidine 5-nucleotidase deficiency Red blood cells glycolysis defects Severe liver failure (all causes) Transaldolase deficiency **Wilson disease** Wolman disease **X-Linked sideroblastic anaemia, pyridoxine-responsive**

MLASA, Myopathy, lactic acidosis and sideroblastic anaemia; *IRIDA*, iron-refractory iron-deficiency anaemia. Treatable disorders are shown in **boldface type**

Table 1.35. Disturbances affecting white blood cells and platelets

Pancytopenia – thrombocytopenia – leukopenia	Vacuolated lymphocytes	Miscellaneous
Aspartylglucosaminuria Barth syndrome (neutropenia) CDG IIf (CMP sialic acid transporter) Dursun syndrome (neutropenia) **Gaucher disease type I and III** Niemann-Pick disease type A and B **Glycogenosis type Ib (neutropenia)** **Inborn errors of cobalamin metabolism** **Inborn errors of folate metabolism** **Lysinuric protein intolerance** **Organic acidurias** (methylmalonic, propionic, isovaleric: in acute attacks) All conditions with marked splenomegaly Pearson syndrome Respiratory chain disorders Adenylate kinase 2 deficiency (reticular dysgenesis) Schwachman syndrome Transaldolase deficiency	Aspartylglucosaminuria Austin disease Ceroid lipofuscinosis I-Cell disease (mucolipidosis type II) Landing disease (GM1) Mucopolysaccharidosis Niemann-Pick disease type Ia, c Pompe disease Sialidosis Wolman disease	**Hyperleukocytosis (>100,000):** – Leukocyte adhesion deficiency syndrome (CDG IIc: GDP fucose transporter 1) **Haemophagocytosis:** – CblC – Gaucher disease – Lysinuric protein intolerance – Niemann-Pick disease

Treatable disorders are shown in **boldface type**

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 37).

▪ White Blood Cells and Platelets Disturbances

These are summarised in ◘ Table 1.35. Dursun syndrome with congenital neutropenia and mutations in G6PC3 has been elucidated recently [51, 52].

1.6.7 Hepatology

- **Cholestatic Jaundice and Cirrhosis**

For information on these topics the reader is referred to ◘ Table 1.36.

- **Liver Failure (Ascites, Oedema)**

For more on this topic see ► Section 1.4.1 and ◘ Table 1.13.

- **Hepatomegaly and Hepatosplenomegaly** (see also ► Section 1.3.1)

Hepatomegaly in inborn errors of metabolism occurs in three different circumstances:

1. With manifestations of hepatocellular necrosis (► Section 1.4.1 and ◘ Table 1.13)
2. With cholestatic jaundice (◘ Table 1.36)
3. With no prominent hepatic dysfunction

When hepatomegaly is the main or only presenting symptom of liver disease, a major clinical clue to the diagnosis is the consistency of the liver and the characteristics of its surface. A firm or rock-hard consistency may indicate tyrosinaemia type I, galactosaemia, glycogenosis type IV, severe neonatal haemochromatosis, alpha 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, the lysosomal disorders should be considered: coarse facies, bone changes, joint stiffness, ocular symptoms, vacuolated lymphocytes and neurological deterioration are strongly suggestive of the mucolipidoses and mucopolysaccharidoses. Failure to thrive, anorexia, poor feeding, severe diarrhoea, 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, glycogenosis type Ib and lysinuric protein intolerance. Hepatosplenomegaly can be the presenting sign in Gaucher disease type I and in Niemann-Pick disease type Ib (with asymptomatic interstitial pneumonia in the latter). In late infancy or childhood, hepatosplenomegaly associated with myoclonic jerks, ophthalmoplegia and neurological deterioration strongly suggests the diagnosis of late-onset forms of Niemann-Pick disease type C and subacute neuronopathic Gaucher disease type III.

When hepatomegaly is not associated with splenomegaly, three clinical circumstances should be considered. Situations with fasting hypoglycaemia suggest glycogenosis 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); these pa-

◘ **Table 1.36.** Cholestatic jaundice and cirrhosis

Cholestatic jaundice	Cirrhosis
α-1-Antitrypsin deficiency	Alpers progressive infantile poliodystrophy
Arginase deficiency	α-1-Antitrypsin deficiency
Bile acid metabolism defects	**Arginase deficiency**
Byler disease	CDG
CDG Cerebrotendinous xanthomatosis	Cholesterol ester storage disease
Cholesterol synthesis defects	Cystic fibrosis
Citrin deficiency	**CDG Ib**
COG 7 deficiency (CDG type II)	**Galactosaemia**
Cystic fibrosis	**Gaucher disease**
Galactosaemia	Glycogenosis type IV
LCHAD deficiency	Haemochromatosis
Methylacyl CoA racemase deficiency	**Hereditary fructose intolerance**
Mevalonic aciduria	**Hypermanganesaemia with dystonia**
Niemann-Pick disease type C	Indian childhood cirrhosis: Idiopathic copper toxicosis
Peroxisomal disorders	**LCHAD deficiency**
Transaldolase deficiency	Niemann-Pick disease
Tyrosinaemia type I	Peroxisomal disorders
	S-Adenosine homocysteine hydrolase deficiency
	Transaldolase deficiency
	Tyrosinaemia type I
	Wilson disease
	Wolman disease

Treatable disorders are shown in **boldface type**

tients have a doll-like appearance and short stature. Fructose 1,6-biphosphatase deficiency is considered when hypoglycaemia 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 glycogenosis. Isolated hepatomegaly with a protuberant abdomen is a presenting sign of glycogenosis type VI and IX (phosphorylase and phosphorylase b kinase) and is also observed in the rare entities cholesteryl ester storage disease, Tangier disease and neutral lipid storage disorders.

1.6.8 Immunology

- **Inflammatory Syndrome**

Inflammatory syndrome may be a presenting preponderant sign in the following three disorders:
- Aicardi Goutières syndrome (altered cytokine expression) [22, 23] (► Section 1.6.16)
- Hyper-IgD syndrome
- Mevalonate kinase deficiency

- **Macrophage-activating Syndrome**

Macrophage-activating syndrome may be observed in the following four disorders:
- **Gaucher disease**
- **Lysinuric protein intolerance**
- Niemann-Pick disease
- **Propionic acidaemia**

- **Severe Immune Deficiency (Affecting T Cells, B Cells or Combined)**

Severe Immune Deficiency is a presenting or associated sign in the following disorders:
- Abetalipoproteinaemia (altered presentation of self- and microbial lipid antigens by CD1 molecules) [53]
- Adenosine deaminase deficiency (severe combined)
- Adenylate kinase 2 (reticular dysgenesis)
- **Hereditary orotic aciduria** (**combined**)
- **Lysinuric protein intolerance** (low CD4+ Tcells)
- Purine nucleoside phosphorylase (T cell)
- **Transcobalamin II** (B cells: hypogammaglobulinaemia)

1.6.9 Myology

Many IEM can present with severe hypotonia, muscular weakness, and poor muscle mass. These include most of the late-onset forms of urea cycle defects and many organic acidurias. Severe neonatal generalised hypotonia plus 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 respiratory chain disorders and other congenital hyperlactataemias, FAO defects, peroxisomal disorders, muscular glycogenolysis defects, alpha glucosidase deficiency and some other lysosomal disorders (► Section 1.3.1). Hypotonia, generalised weakness, reduced muscle mass and developmental delay are also the presenting features of the Allan-Herndon-Dudley syndrome caused by the monocarboxylate transporter 8 mutations, which involves the transport of tri-iodothyronine into neurons and disturbs blood levels of thyroid hormone [48].

- **Exercise Intolerance, Myoglobinuria, Cramps, Muscle Pain, Elevated CK**

See ► Section 1.4.1 for acute symptoms.

- **Myopathy (Progressive)**

Many metabolic myopathies have been described but only a few are effectively treatable:
- Muscle adenylate deaminase deficiency
- **FAO disorders**
- **ETF deficiency and ETF dehydrogenase deficiency**
- Glycogenosis type II (acid maltase deficiency)
- Glycogenosis type III
- Multisystemic triglyceride storage disease
- Respiratory chain disorders (Kearns-Sayre syndrome and many others)
- Steinert disease

1.6.10 Nephrology

This topic is summarised in ◘ Table 1.37. A specific defect involving NaPi-IIa implicated in phosphate reabsorption has recently been described [54].

1.6.11 Neurology and Psychiatry

For information on this topic see ► Section 1.5.

1.6.12 Ophthalmologic Signs

See also ► Section 1.5.2, 'Neuro-ophthalmologic Signs'.

- **Cataracts**

Metabolic causes of cataracts are presented according to age of onset in ◘ Table 1.38.

Table 1.37. Main presentations in nephrology

Nephrolithiasis/nephrocalcinosis (composition)	Polycystic kidneys	Tubulopathy	Urines (colour, odour)	Miscellaneous
APRT deficiency (2-8 dihydroxy adenine) **Cystinuria (cystine)** **Glycogenosis type I** (uric acid) Hereditary hyperparathyroidism (calcium) Hereditary renal hypouricaemia (uric acid) Hypomagnesaemia with hypercalciuria and nephrocalcinosis (calcium) **Hyperoxaluria type I, II, III** (oxalic) Lesch-Nyhan (uric acid) Molybdenum co-factor deficiency (xanthine) PRPP synthase superactivity (uric acid) **Renal tubular acidosis type I** Uric acid transporter defects (uric acid) **Xanthine oxidase deficiency** (xanthine) Familial juvenile hyperuricaemic nephropathy (uric acid, uromodulin) **Wilson disease** (calcium)	CDG CPT II deficiency Glutaric aciduria type II Zellweger syndrome	**Fanconi syndrome:** – Cystinosis – **Hereditary fructose intolerance – galactosaemia** – **Fanconi Bickel-syndrome:** GLUT II – Lowe syndrome (X-linked *OCRL*1) – NaPi-IIa (phosphate reabsorption) – Respiratory chain disorders (complex III, IV, mitochondrial DNA deletion, depletion) – **Tyrosinaemia type I** – **Wilson disease** **Renal tubular acidosis:** – Carbonic anhydrase II (with osteopetrosis, proximal) – **RTA type I** (isolated, distal) – RTA type II (isolated, proximal) – PC deficiency (proximal) – **Methylmalonic aciduria** – **Glycogenosis type I** – **CPT I deficiency (combined)** – Dent disease (*CLCN5* mutations) **Hypochloraemic alkalosis:** – Bartter and Gitelman syndromes – Congenital chloride diarrhoea – Mitochondrial seryl-tRNA synthetase defect (HUPRA syndrome)	**Abnormal colour:** – Alkaptonuria (black) – Indicanuria (blue) – Myoglobinuria (red) – Porphyria (red) **Abnormal odour:** – Dimethylglycine dehydrogenase (fish) – **3-CH3-crotonylglycinuria (cat)** – Glutaric aciduria type II (sweaty feet) – **Isovaleric acidaemia** (sweaty feet) – **MSUD** (maple syrup) – **Phenylketonuria** (musty) – Trimethylaminuria (fish) – **Tyrosinaemia type I** (boiled cabbage)	**Haemolytic uraemic syndrome:** – **Inborn errors of cobalamin metabolism** (Cbl C, Cbl G) **Nephrotic syndrome:** – Respiratory chain disorders **Nephropathy (tubulointerstitial)** – **MMA aciduria** – Respiratory chain disorders (pseudo-Senior-Loken syndrome)

APRT, Adenine phosphoribosyl transferase. Treatable disorders are shown in **boldface type**

Table 1.38. Cataracts

Detectable at birth (congenital)	Detectable in the newborn period (1st week to 1st month)	Detectable in infancy (1st months to 1st years)	Detectable in childhood (1-15 years)	Detectable in adulthood (>15 years)
Cockayne syndrome Lowe syndrome (*OCRL1* X-linked mutation) Peroxisomal biogenesis defects (Zellweger and variants) Phosphoglycerate dehydrogenase deficiency Rhizomelic chondrodysplasia punctata Sorbitol dehydrogenase deficiency	**Galactosaemias** Marginal maternal galactokinase deficiency Peripheral epimerase deficiency (homozygotes and heterozygotes)	Alpha mannosidosis Galactitol or sorbitol accumulation of unknown origin **Galactokinase deficiency** Hypoglycaemia (various origins/disorders) P5C synthase deficiency Respiratory chain disorders Sialidosis Copper chaperone CCS (that delivers Cu to both copper/zinc SOD1 and XIAP (X-linked inhibitor of apoptosis)	**Diabetes mellitus** Dominant cataract with high serum ferritin **Hypoparathyroidism** **Lysinuric protein intolerance** Mevalonic aciduria Neutral lipid storage disorder PHARC syndrome Pseudo-hypoparathyroidism Sjögren-Larsson syndrome **Wilson disease**	Carriers for Lowe syndrome **Cerebrotendinous xanthomatosis** **Fabry disease** Glucose-6-phosphate dehydrogenase deficiency **Heterozygotes for GUT and galactokinase** **Homocystinurias** **Lactose malabsorbers** Mevalonate kinase Mitochondrial cytopathies **Ornithine aminotransferase deficiency** PEX 7 Refsum disease Steinert dystrophy (cataract can be presenting sign) **Tangier disease**

GUT, Galactose uridyltransferase. Treatable disorders are shown in **boldface type**

■ **Corneal Opacities (Clouding)**

Metabolic causes of corneal opacities are presented according to age of onset in ◘ Table 1.39.

■ **Ectopia Lentis (Dislocation of the Lens)**

A search for ectopia lentis is mandatory for the following disorders:

- **Classic homocystinuria** (downward dislocation)
- Sulfite oxidase deficiency and molybdenum cofactor deficiency
- Marfan syndrome (upward dislocation)
- Marchesani syndrome

■ **Keratitis, Corneal Opacities** (see also ◘ Table 1.39)

These are presenting signs of the following two treatable disorders:

- **Tyrosinaemia type II**
- **Fabry disease** (**X-linked**)

■ **Microcornea**

- Ehlers Danlos disease type IV

1.6.13 Osteology

For information on this topic the reader is referred to ◘ Table 1.40.

1.6.14 Pneumology

■ **Hyperventilation Attacks**

- **Hyperammonaemias**
- Joubert syndrome
- Leigh syndrome (idiopathic or due to various inborn errors)
- **Metabolic acidosis**
- Rett syndrome (girls only)

■ **Pneumopathy (Interstitial)**

Interstitial pneumopathy is a frequent complication and may occasionally be a presenting sign in the following disorders:

◘ **Table 1.39.** Corneal clouding

Visible in early infancy (3-12 months)	Visible in late infancy to early childhood (1-6 years)	Visible in late childhood, adolescence to adulthood:
Tyrosinaemia type II (presenting sign) **Cystinosis** (presenting sign) Galactosialidosis Hurler, Sheie disease(MPS I) I-Cell disease (mucolipidosis type II) Infantile free sialic acid storage disease Maroteaux-Lamy syndrome (MPS VI) Steroid sulfatase deficiency	Mucolipidosis type IV (presenting sign) Alpha-mannosidosis (late-onset form) Lecithin cholesterol acyltransferase deficiency Morquio disease (MPS IV) Pyroglutamic aciduria (presenting sign) MPS I H and VII **Tangier disease**	**Fabry disease** (X-linked) Galactosialidosis (juvenile form) **Wilson disease** (green Kaiser-Fleischer ring)

Treatable disorders are shown in **boldface type**

◘ **Table 1.40.** Osteology

Osteopenia	Punctate epiphyseal calcifications	Exostosis (hereditary multiple)
Cerebrotendinous xanthomatosis (and several other types of cholesterol synthesis defects) CDG **Galactosaemia** **Glycogenosis type I** **Homocystinuria** I-Cell disease (MLD type II) Infantile Refsum disease **Lysinuric protein intolerance** **All organic acidurias** (chronic forms)	Beta glucuronidase deficiency Chondrodysplasia punctata, rhizomelic type Conradi Hunermann syndrome Familial resistance to thyroid hormone Peroxisomal disorders (Zellweger and variants) Spondylo-enchondromatosis Warfarin embryopathy	*O*-Glycosylation defects (EXT1-EXT2) Occipital horn syndrome

Treatable disorders are shown in **boldface type**

- **Gaucher disease**
- **Lysinuric protein intolerance**
- Niemann-Pick disease type B

- **Stridor**
- **Biotinidase deficiency**
- **Hypocalcaemia**
- **Hypomagnesaemia**
- **Multiple acyl-CoA dehydrogenase deficiency** (**riboflavin responsive**)
- Pelizaeus-Merzbacher disease

- **Pulmonary Hypertension**
- Dursun syndrome (with neutropenia) [51, 52]
- **Glycogenosis type I**
- Nonketotic hyperglycinaemia
- HUPRA syndrome [31]

1.6.15 Psychiatry

See ► Sections 1.4.1 and 1.5.1.

1.6.16 Rheumatology

- **Arthritis – Joint Contractures – Progressive Ankylosis – Bone Necrosis**
- Aicardi-Goutières syndrome (chronic progressive deforming arthropathy with chilblains and contractures) [22, 23]
- Alkaptonuria
- **ANKH mutations** (progressive ankylosis with deafness, mental retardation and hypophosphataemia) [55]
- 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 [56]
- PRPP synthetase, HGPRT
- Uromoduline mutation (familial hyperuricaemic nephropathy)

- **Bone Crisis**

See ► Section 1.4.1.

1.6.17 Stomatology

- **Glossitis, Stomatitis, Mouth Ulcers**
- Aicardi-Gouttières syndrome, [22, 23].
- **Cbl F defect**
- **Folate malabsorption**
- **Intrinsic factor deficiency**
- **Transcobalamin II deficiency**

- **Macroglossia**
- Beckwith-Wiedemann syndrome
- Congenital muscular dystrophies (DMC1C)
- Complex IV deficiency
- Pompe disease

- **Hypodontia**
- Leukoencephalopathy with ataxia [57]
- 4H syndrome [44]

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

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2.1 Introduction

Late-onset forms of IEM presenting initially in adulthood are often unrecognised, so that their exact prevalence is unknown. 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 [1-3].

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.

2.2 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 always 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.

◘ **Table 2.1.** Striking clinical and radiological differences between childhood-onset and adult-onset forms of inborn errors of metabolism

Disease	Classic presentation in childhood	Milder adult-onset forms
Adrenoleukodystrophy	Early-onset dementia, visual and hearing impairment, quadriplegia and cerebellar ataxia, leukodystrophy	Chronic spastic paraparesis, psychiatric features, adrenal insufficiency (Addison disease), peripheral neuropathy. MRI: can be normal or shows a restricted leukoencephalopathy
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, glomerulonephritis, haemolytic uraemic syndrome. MRI: normal or leukoencephalopathy, macrocytic anaemia is rare
Coenzyme Q10 deficiency	Leigh syndrome, myoglobinuria, encephalopathy	Cerebellar ataxia, myopathy
CTX	Mental retardation, chronic diarrhoea, epilepsy, juvenile cataract, neonatal cholestasis	Tendon xanthomas, cerebellar ataxia, spastic paraparesis, dementia, psychiatric signs
Cystathionine synthase deficiency	Mental retardation, epilepsy, autism, lens dislocation, scoliosis	Strokes (internal carotid dissection), deep vein thrombosis, psychiatric disorders

Table 2.1. *Continued*

Disease	Classic presentation in childhood	Milder adult-onset forms
Fatty acid β-oxidation defects	Nonketotic hypoglycaemia, cardiomyopathy, liver disease, rhabdomyolysis, peripheral neuropathy	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 mild mental retardation, macrocephaly, cephalalgia or epilepsy
Glycogenosis type IV (glycogen branching enzyme deficiency)	Liver storage disease	Myopathy (amylopectinosis) or polyglucosan body disease: spastic paraparesis, peripheral neuropathy, leukoencephalopathy with brain stem and 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 motoneuron disease, cerebellar ataxia, dystonia, sensory polyneuropathy
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
Metachromatic leukodystrophy	Progressive gait problems, hypotonia, peripheral neuropathy, spasticity in all four limbs, optic atrophy, cerebellar ataxia	Psychiatric phenotype: »psychosis-like features (mimics schizophrenia), cognitive troubles« Motor phenotype: »spastic paraparesis, cerebellar ataxia, dystonia, demyelinating polyneuropathy«
3-Methylglutaconic aciduria type I	Mental retardation or motor delay, movement disorders, febrile seizures	Ataxia, dementia, optic atrophy, spasticity, leukoencephalopathy
Methylmalonic aciduria	Comas, failure to thrive	Optic atrophy, end stage renal failure
MSUD	Comas, failure to thrive	Episodes of nausea, vomiting, encephalopathy triggered by high protein catabolism
MTHFR deficiency	Progressive encephalopathy with apnoea, epilepsy, microcephaly	Psychiatric disorders, coma, paraplegia, thromboembolic events, polyneuropathy. MRI: leukoencephalopathy
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
NKH	Epilepsy with suppression bursts, encephalopathy	Paroxysmal choreic movement disorders, confusion triggered by fever; mental retardation with aggressive behaviour

Table 2.1. *Continued*

Disease	Classic presentation in childhood	Milder adult-onset forms
PDH deficiency	Lactic acidosis, corpus callosum agenesis, Leigh syndrome, polyneuropathy	Episodic ataxia triggered by fever or carbohydrates, optic neuropathy, dystonia, Parkinsonism, 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
PKU (untreated)	Mental retardation, autistic behaviour, seizures, movement disorders	Spastic paraparesis, optic atrophy, dementia, Parkinsonism
Propionic aciduria	Comas, failure to thrive	Chorea, dementia, acute episodes of nausea or lethargia, chronic cardiomyopathy
Thiamine transporter (SLC19A3) mutations	Biotin responsive basal ganglia disease (encephalopathy, coma, epilepsy, generalised dystonia)	Wernicke-like encephalopathy
Serine deficiency	Mental retardation, epilepsy, microcephaly	Polyneuropathy, ichthyosis, cataract
Sialidosis type 1	Dysmorphic features, mental retardation, progressive encephalopathy	Action and stimulus-sensitive myoclonus, tonic-clonic seizures, macular cherry red spot, cerebellar ataxia
SSADH deficiency	Epileptic encephalopathy	Behavioural/psychiatric disorders, isolated seizures
Urea cycle disorders	Comas, failure to thrive	Nausea, vomiting, cephalalgia, confusion, psychiatric disorders, ataxia, stroke-like episodes, coma
Wilson's disease	Hepatic failure	Psychiatric signs, tremor, parkinsonism, dystonia, dysarthria

Treatable disorders are shown in **boldface type**

2.3 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.

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-partal status; 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 systems (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 sections and tables below.

Metabolic diseases involving the nervous system can be divided into five main categories, all of which dis-

Table 2.2. Examples of syndromes or signs with very high diagnostic value

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
Brain MRI	
Bilateral necrosis of basal ganglia (Leigh syndrome)	Energy metabolism defects (pyruvate dehydrogenase, respiratory chain, coenzyme Q10)
Abnormally low signal of basal ganglia	Iron storage disorders
Abnormally high signal of basal ganglia on T_1-weighted sequences	Disorders of manganese metabolism, porto-systemic shunts
Ophthalmological	
Supranuclear gaze palsy	Lysosomal diseases (Gaucher, Niemann Pick C)
Bilateral optic neuropathy	Energy metabolism defects (pyruvate dehydrogenase, respiratory chain)
Macular cherry red spot	Sialidosis
Cutaneous	
Progressive dysmorphia	Lysosomal diseases
Angiokeratoma	Lysosomal diseases
Xanthomata (Achilles tendons)	Cholesterol metabolism defects (cerebrotendinous xanthomatosis)
Ichthyosis	Disorders of lipid metabolism (Sjögren Larsson syndrome, Refsum's disease)
Visceral	
Splenomegaly	Lysosomal diseases, Tangier disease
Venous and arterial thrombosis	Homocystinurias
Gout, nephrolithiasis, tophi	Purine salvage (Lesch-Nyhan syndrome)
Past history of neonatal cholestatis	Disorders of cholesterol metabolism (Niemann-Pick C, hereditary spastic paresis type SPG5, cerebrotendinous xanthomatosis, alpha-methyl-acyl-CoA racemase deficiency), citrin deficiency

play some similarities in clinical presentation, diagnostic methods and treatment strategies.

2.3.1 Energy Metabolism Defects

Energy metabolism defects 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 or pseudo-strokes. Chronic presentations often involve muscles, cerebellum, basal ganglia (parkinsonism), cortex (epilepsy, myoclonus) or the peripheral nervous system (axonal polyneuropathy). In adults, these diseases rarely involve the brain white matter and spastic paraparesis is very uncommon.

2.3.2 Disorders of Lipid Metabolism

Lipid metabolism disorders include some lysosomal diseases, mainly sphingolipidoses (Krabbe's disease, metachromatic leukodystrophies, Niemann Pick A, B and C, Fabry disease and Gaucher disease), peroxisomal disorders (adrenomyeloneuropathy, Refsum disease, disorders of pristanic acid metabolism, peroxisome biogenesis disorders), Tangier disease and cerebrotendinous xanthomatosis. Given the huge proportion of lipids in the nervous system, these diseases can produce almost all kinds of symptoms. Leukodystrophies and demyelinating polyneuropathies are hallmarks of disorders interfering with myelin formation or maintenance. A past history of prolonged neonatal jaundice is suggestive of disorders of cholesterol and bile acid metabolism. Splenomegaly is highly suggestive of some lipid storage diseases, such as Niemann-Pick C, Gaucher disease, Tangier disease and Niemann Pick B. Other presentations are less specific: cerebellar ataxias, dementia, psychiatric disorders, epilepsy, spastic paraparesis. Slow progression of symptoms, corresponding to progressive lipid storage, is highly suggestive of these disorders.

2.3.3 Intoxication Syndromes

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

2.3.4 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. Cerebral folate deficiency can be added to this group because it shares several clinical features and diagnostic methods although this syndrome is still highly heterogeneous.

2.3.5 Metal Storage Disorders

Metal storage disorders include Wilson's disease, neuroferritinopathy, aceruloplasminaemia, PANK2-associated neurodegeneration, PLA2G6 mutations 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. 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.

2.4 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.

2.4.1 Encephalopathies/Comas [4]

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.

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 almost always abnormal, showing bilateral lesions of basal ganglia (Leigh syndrome or stroke-like lesions). Thus, the diagnostic approach is mainly based on MRI.

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

Table 2.3. Diagnostic approach to metabolic causes of encephalopathies/strokes

Diseases	Encephalopathy/coma	Strokes or pseudo-strokes
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	+	
NKH	+	
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; *RD*, remethylation defects. Treatable disorders are shown in **boldface type**

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 thalami and brain stem, with cortical lesions mimicking infectious encephalitis or pseudo-strokes.

2.4.2 Strokes and Pseudo-strokes

Some IEM cause ischaemic strokes in adulthood. 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. Recently, ischaemic brain lesions have also been reported in patients with Pompe disease. In addition, acute focal neurological signs mimicking strokes (pseudo-strokes) can be seen in patients with urea cycle disorders, mitochondriopathies 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 pseudo-strokes from true ischaemic strokes is diffusion imaging: the diffusion coefficient is typically normal or increased in the former and decreased in the latter.

2.4.3 Movement Disorders [5]

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 basal ganglia are observed on brain MRI (Table 2.4 and Table 2.5).

Generally, a particular movement disorder can be seen in many different IEM, and conversely, a given

Table 2.4. Diagnostic approach to metabolic causes of movement disorders

Diseases	Parkinsonism	Dystonia	Chorea	Myoclonus	Paroxysmal dystonia
Energy metabolism disorders					
Respiratory chain disorders	+	+	+	+	
PDH deficiency	+	+	+		+
GLUT1 deficiency		+	+		+
BBGD		+			
Vitamin E deficiency		+			
Lipid metabolism disorders					
CTX	+				
Niemann-Pick type C	+	+	+	+	
GM1 gangliosidosis	+	+			
GM2 gangliosidosis	+	+	+		
Gaucher disease	+	+		+	
Intoxication syndromes					
Phenylketonuria	+				
Homocystinuria	+	+	+		
L-2-Hydroxyglutaric aciduria	+	+			
Propionic acidaemia			+		
Neurotransmitter metabolism defects					
GTP cyclohydrolase-1 deficiency	+	+			+
Tyrosine hydroxylase deficiency	+	+			
Dopamine transporter deficiency	+	+			
PTP synthase deficiency		+			+
Sepiapterin reductase deficiency	+	+			
Nonketotic hyperglycinaemia			+		
Metal storage disorders					
Wilson's disease	+	+	+		+
Aceruloplasminaemia	+	+	+		
Panthotenate kinase deficiency	+	+	+		
Neuroferritinopathy	+	+	+		
Manganese metabolism disorder	+	+			
Calcium-independent phospholipase A2 (PLA2G6) mutations	+	+			
Others					
Ceroid-lipofuscinosis	+	+		+	
Lesch-Nyhan		+	+		
Sialidosis				+	

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			+	+	
PDH deficiency	+	+	+	+	+
Co-enzyme Q10 deficiency			+	+	
Mitochondrial thiamine pyrophosphate transporter (SLC25A19)			+		
Lipid storage					
Cerebrotendinous xanthomatosis	+				+
AMACR deficiency		+		+	
GM1 gangliosidosis			+		
Fabry disease		+			
Intoxication syndromes					
Methylmalonic aciduria	+				
SSADH deficiency	+				+
Urea cycle disorders	+				+
Glutaric aciduria type 1			+		
Metal storage disorders					
Wilson disease	+	+	+	+	+
Aceruloplasminaemia	+	+	+	+	+
Neuroferritinopathy	+		+	+	+
PANK2	+				
PLA2G6	+			+	
Hypermanganesaemia	+		+		+

Treatable disorders are shown in **boldface type**

IEM can present with different abnormal movements. As a consequence, the classic phenomenological diagnostic approach to movement disorders (i.e. dystonia, parkinsonism, chorea, mycolonus) is less applicable to the diagnosis of IEM. As in the case of acute encephalopathies, the brain MRI plays a central role in the diagnostic approach.

Necrotic lesions of basal ganglia suggest an energy metabolism disorder (see above). Reduced signal intensity of the pallidum suggests iron storage disorders (PANK2 mutations, neuroferritinopathy, PLA2G6 mutations, aceruloplasminaemia). The pattern of these lesions within the basal ganglia may help to differentiate between these particular disorders (Table 2.5) [6].

Diffuse high signals involving thalami, brain stem and cerebellar peduncles are highly suggestive of Wilson's disease.

When MRI is normal, the diagnostic approach can be based on the course of the disease. Progressive neurodegeneration suggests a lysosomal storage disease such as Niemann-Pick type C and GM1 and GM2 gangliosidoses. In contrast, non progressive parkinsonism partially respon-

sive to levodopa is typically observed in some respiratory chain disorders such as POLG mutations and complex 1 deficiency. 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.

2.4.4 Peripheral Neuropathies [7]

Peripheral neuropathies in the context of IEM are often labelled 'Charcot-Marie-Tooth disease'. These types of neuropathies are characterised by long-standing chronic, predominantly motor, distal and symmetrical polyneuropathy with pes cavus, 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). However, in some cases, peripheral neuropathies may be acute or relapsing, and may involve multiple nerves (mononeuropathy, multiplex), motoneurons or dorsal root ganglia; ◘ 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 is typically responsible for a demyelinating polyneuropathy. Some lipid storage disorders, such as cerebrotendinous xanthomatosis, adrenomyeloneuropathy and other peroxisomal diseases, may cause polyneuropathies that can be axonal, demyelinating or both. Recently, an autosomal dominant form, a hereditary neuropathy involving unmyelinated sensory fibres and motor neurons (hereditary sensory and autonomic neuropathy type 1), has been linked to mutations in the serine palmitoyltransferase, which catalyses the first step of sphingolipids synthesis, leading to the toxic accumulation of abnormal sphingolipids [8] (◘ Chapter 35). Acute polyneuropathies mimicking Guillain-Barré syndrome can be observed in acute attacks of porphyria and in pyruvate dehydrogenase deficiency, acute exacerbations of Refsum's 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-Sachs disease.

Lastly, involvement of dorsal root ganglia is highly suggestive of POLG mutations (mtDNA polymerase ɣ). 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.

2.4.5 Leukoencephalopathies [9-11]

Determining the cause of a leukoencephalopathy is often a diagnostic challenge. The first step in the diagnostic approach 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.

The diagnostic approach to genetic leukoencephalopathies should be guided by the clinical examination, the MRI aspect and electroneuromyographic studies. Each IEM is 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.

2.4.6 Epilepsy [12]

Although epilepsy is a frequent presentation of IEMs 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.

Table 2.6. Diagnostic approach to metabolic causes of peripheral nervous system

Diseases	Demyelinating	Axonal	Mn	Small fibres	DRG	Acute	Mono-neuropathy multiplex
Energy metabolism disorders							
Respiratory chain disorders		+			+		
MNGIE	+						
PDH deficiency		+				+	
Vitamin E deficiency		+					
β-Oxidation defects		+					
Biotinidase deficiency			+			+	
Lipid storage/oligosaccharidoses							
CTX	+	+					
GM2 gangliosidosis			+	+			
Fabry disease				+			
Metachromatic leukodystrophy	+						
Krabbe disease	+	+					
Adrenoleukodystrophy	+	+					
Refsum disease	+					+	+
AMACR deficiency	+	+					
Peroxisome biogenesis defects	+	+					
Tangier disease				+		+	+
β-Mannosidosis	+						
Serine palmitoyltransferase mutations		+	+	+			
Others							
Serine deficiency		+					
Homocysteine RD	+	+			+		
Polyglucosan body disease		+	+				
Acute porphyrias		+		+		+	+
CDG syndromes		+					
Tyrosinaemia type 1						+	

DRG, dorsal root ganglia; *Mn*, motoneuron involvement; *MNGIE*, mitochondrial neurogastro intestinal encephalo myopathy; *RD*, remethylation defects. Treatable disorders are shown in **boldface type**

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 re-

Table 2.7. Diagnostic approach to metabolic causes of leukoencephalopathies

Diseases	Periventricular	Pyramidal tracts	Cerebellum	Spinal cord	Juxta-cortical	Brain stem	Corpus callosum
Lipid storage							
Metachromatic leukodystrophy	+						+
Adrenoleukodystrophy		+					+
Krabbe disease		+					+
CTX	+	+	+	+		+	+
Refsum's disease	+						
AMACR deficiency						+	
PBD	+	+				+	
α-Mannosidosis	+						
Niemann-Pick C	+						
Intoxication syndromes							
Homocysteine RD	+			+	+		
Phenylketonuria	+						+
Glutaric aciduria type 1	+				+		
L-2-(HO)-glutaric aciduria					+		
3-Methylglutaconic aciduria					+		
Energy metabolism disorders							
RCD	+	+	+	+	+	+	+
APGBD	+	+	+	+	+	+	
MNGIE	+				+		
DARS2 mutations	+	+	+	+		+	
Others							
Wilson disease			+			+	

APBGD, adult polyglucosan body disease; *RCD*, respiratory chain disorders; *MNGIE*, Mitochondrial neurogastroIntestinal encephalomyopathy. Treatable disorders are shown in **boldface type**

lated 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).

The three main groups of IEM presenting with epilepsy in adults are disorders of energy metabolism, intoxications and lysosomal diseases (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.

2.4.7 Psychiatric Disorders [13, 14]

IEM frequently present with psychiatric diseases in adolescents or adults. Retrospective analysis of patients with various IEM shows that psychiatric signs may remain

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 and others)	+	+
GAMT deficiency	+	
GLUT1 deficiency	+	+
SLC19A3 mutations	+	
Lipid metabolism/storage		
CTX	+	
Niemann-Pick C	+	+
Gaucher type 3	+	+
Ceroid lipofuscinosis	+	+
LIMP2 deficiency	+	+
Sialidosis	+	+
Lafora disease		
Intoxication syndromes		
Homocysteine remethylation defects	+	
L-2-Hydroxyglutaric aciduria	+	
CBS deficiency	+	
SSADH deficiency	+	
Acute intermittent porphyrias	+	
Lysinuric protein intolerance	+	
Others		
Hyperinsulinism-hyperammonaemia	+	+

SLC, thiamine transporter; *GAMT*, guanidinoacetate N methyl transferase; *LIMP2*, lysosomal integral membrane protein type 2; *SSADH*, succinate semialdehyde dehydrogenase. Treatable disorders are shown in **boldface type**

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 probably 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.

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,

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 defciency		+
Intoxication syndromes		
Urea cycle disorders	+	+
Homocysteine remethylation defects	+	+
CBS deficiency	+	+
cute intermittent porphyrias	+	
NKH		+
SSADH deficiency		+
Phenylketonuria	+	+
Lipid storage/oligosaccharidoses/MPS		
Niemann-Pick C[1]	+	
GM2 gangliosidosis	+	
Metachromatic leukodystrophy	+	
Adrenoleukodystrophy	+	
Cerebrotendinous xanthomatosis	+	+
β-Mannosidosis		+
α-Mannosidosis	+	+
Ceroid lipofuscinoses	+	+
San Filipo syndrome		+
AMACR deficiency	+	
Metal storage disorders		
Wilson disease	+	
Aceruloplasminaemia	+	
Neuroferritinopathy	+	
PANK2	+	+

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

alteration of consciousness). This group corresponds to intoxications (mainly urea cycle defects, homocysteine remethylation defects and porphyrias).

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 homocystinurias (homocysteine re-methylation defects and cystathionine β-synthase deficiency), iron storage disorders and lipid metabolism disorders (metachromatic leukodystrophy, GM2 gangliosidosis, Niemann-Pick type C disease, adrenoleukodystrophy and cerebrotendinous xanthomatosa). Patients in this group may initially present with recurrent psychotic attacks, chronic delusion or disorganised behaviour, which may mimic schizophrenia.

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

Diseases	Chronic	Acute
Lipid metabolism		
CTX	+	
Adrenoleukodystrophy	+	
Krabbe disease	+	
Intoxication syndromes		
Phenylketonuria	+	
Arginase defiency	+	+
Triple H syndrome	+	
Homocysteine remethylation defects	+	+
L-2-Hydroxyglutaric aciduria	+	
Neurotransmitter metabolism defects		
GTPCH1 deficiency	+	
Tyrosine hydroxylase deficiency	+	
Others		
Biotinidase deficiency		+
Polyglucosan body disease	+	

ctx, cerebrotendinous xanthomatosis. Treatable disorders are shown in **boldface type**

It also includes behavioural and personality changes. The diagnosis is particularly difficult in this group given the relative nonspecificity of psychiatric signs, especially when they remain isolated for years or decades. However, catatonias, visual hallucinations, 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, nonketotic hyperglycinaemia, 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, San Filippo syndrome).

Given the important number of IEM presenting with psychiatric problems, minimal investigations include brain MRI, ophthalmological examination, abdominal ultrasonography and electromyogram. Furthermore, measurement of plasma homocysteine is always mandatory.

2.4.8 Spastic Paraparesis

Spastic paraparesis is a general term describing progressiv 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 45 different genetic loci or HSP have been identified, with various modes of inheritance [15]. HSP are clinically classified as 'uncomplicated' (or 'pure') when symptoms are limited to spastic paraparesis and as 'complicated' (or 'syndromic') when other neurological or systemic signs do exist.

However, although poorly recognised by neurologists, spastic paraparesis is also one of the many presentations of IEM in children and adults [16]. 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 highly 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.

2.4.9 Cerebellar Ataxia

Cerebellar ataxia is a difficult challenge in neurology (Table 2.11). Except for focal cerebellar lesions, the many causes of cerebellar ataxia include inflammatory diseases, paraneoplastic disorders, acquired metabolic disorders, alcohol intoxication, multiple system atrophy, genetic diseases (Friedreich ataxia, dominant spinocerebellar ataxias etc.).

Cerebellar ataxia in the context of IEM may be acute, triggered by fever (PDH deficiency, respiratory chain disorders or SLC19A3 mutations [17], or chronic. Chronic cerebellar ataxia is rarely pure. In practice, several situations are usually encountered:

Table 2.11. Diagnostic approach to metabolic causes of cerebellar ataxia

Diseases	Chronic cerebellar ataxia	Spino-cerebellar 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				
CTX	+	+		
Niemann-Pick type C	+			+
GM2 gangliosidosis	+			
Gaucher type 3	+			
Adrenoleukodystrophy		+		
Refsum disease	+			
Acyl-CoA oxidase deficiency	+			
Pex10 mutations	+			
α-Mannosidosis	+			
Sialidosis				+
α-/β-Lipoproteinaemia	+	+		
Intoxication syndromes				
Urea cycle disorders			+	
Urocanase deficiency			+	
Mevalonate kinase deficiency	+			
Others				
Hartnup disease			+	

PEX, Peroxin. Treatable disorders are shown in **boldface type**

- Associated polyneuropathy: 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 spino-cerebellar ataxia) suggests a lipid metabolism disorder (cerebrotendinous xanthomatosis or adrenomyeloneuropathy).
- Isolated cerebellar ataxia can be the only presenting sign of GM2 gangliosidosis or of Niemann-Pick disease type C.

2.4.10 Myopathy

Metabolism within muscle 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 usu-

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	+	+	
Trifunctional protein deficiency		+	
CPT2 deficiency		+	
Primary carnitine deficiency	+		+
Others			
Cystinosis	+		
AGAT deficiency	+		
GAMT deficiency	+		
AMACR deficiency		+	
Neutral lipid storage disorders	+		+

AGAT, l-Arginine glycine amidinotransferase; *AMACR*, α-methyl-acyl-CoA racemase; *CBS*, cystathionine β-synthase; *CDG*, congenital disorders of glycosylation; *GAMT,* guanidinoacetate N-methyltransferase; *MCL*, metachromatic leukodystrophy; *PEO*, Progressive external ophtalmoplegia; *POLG*, mitochondrial DNA polymerase gamma. Treatable disorders are shown in **boldface type**

ally spare the nervous system. Hallmarks of metabolic myopathies are exercise intolerance (exertional cramps or fatigue) and recurrent rhabdomyolysis [18, 19]. 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 three main groups of metabolic diseases affecting the muscle are (1) mitochondrial disorders; (2) fatty acids β-oxidation defects (FAOD) and (3) glycogen storage disorders (GSD).

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 myoglobinuria triggered by prolonged exercise or prolonged fasting, but progressive proximal weakness with lipid storage is also a common presentation in adults.

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).

2.4.11 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	Retinitis	Macula cherry red spot	Optic nerve disorders	Cataract	Gaze palsies
Energy metabolism disorders							
RCD	+		+		+	+	+
Vitamin E deficiency			+				
Biotinidase deficiency	+				+		
PDH deficiency					+		+
SLC19A3 mutations (Wernicke like)							+
Intoxication syndromes							
Homocysteine RD					+		
CBS deficiency						+	
Phenylketonuria					+		
Galactokinase deficiency						+	
Lipid storage							
Niemann-Pick C	+						+
MLD					+		
Adrenoleukodystrophy	+				+		
Gaucher disease							+
Fabry disease	+	+				+	
CTX						+	
Ceroid lipofuscinoses			+		+		
Refsum disease	+		+			+	
AMACR deficiency			+				
Acyl-CoA oxidase deficiency			+			+	
PBD	+		+			+	
MPS	+	+	+			+	

Table 2.13. *Continued*

Diseases	Deafness	Corneal	Retinitis	Macula cherry red spot	Optic nerve disorders	Cataract	Gaze palsies
Fish eye syndrome		+					
Cystinosis		+					
α-Mannosidosis	+						
β-Mannosidosis	+						
Sialidosis type 1				+			
Tangier disease						+	
Metal storage disorders							
Wilson disease		+				+	
Aceruloplasminaemia			+				
PANK2 mutations			+				
Others							
CDG syndrome (Ia)			+				

MCL, metachromatic leukodystrophy. Treatable disorders are shown in **boldface type**

Table 2.14. Diagnostic approach to metabolic causes of miscellaneous presentations

Diseases	Dys-autonomia	Gliomas	Aseptic meningitides	Pseudotumor cerebri	Abdominal pain
Energy metabolism disorders					
RCD	+		+		
MNGIE	+				+
APGBD	+				
Succinate dehydrogenase		+			
2OH glutaric aciduria		+			
Intoxication syndromes					
Acute porphyrias	+				+
Galactokinase deficiency				+	
Lipid storage					
Fabry disease			+		+
GM2 gangliosidosis	+				
Cystinosis	+			+	
Others					
CDG syndrome (Ic)				+	

APGBD, Adult polyglucosan body disease. Treatable disorders are shown in **boldface type**

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Newborn Screening for Inborn Errors of Metabolism

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3.1 Introduction

Newborn screening was first applied to the detection of phenylketonuria (PKU) by a bacterial inhibition assay pioneered in 1961 by Guthrie, who was also responsible for the introduction of the use of a dried blood sample [1]. This was followed by further bacterial inhibition assays to detect other aminoacidopathies (maple syrup urine disease, homocystinuria, urea cycle disorders, etc.), but only screening for PKU was widely adopted. In 1975 Dussault described screening for congenital hypothyroidism (CH) [2], and since then other disorders covered in some screening programmes have included congenital adrenal hyperplasia, the galactosaemias, cystic fibrosis, biotinidase deficiency, glucose-6-phosphate dehydrogenase deficiency and many others. The application of molecular genetic testing to newborn screening was first suggested in 1988 [3] and that of tandem mass spectrometry (MS/MS) in 1990 [4]. The latter technology has greatly changed newborn screening, with rapid simultaneous analysis of multiple analytes, thus allowing the identification of more than 40 inborn errors of amino acid, organic acid and fatty acid metabolism.

This chapter only deals with newborn screening employing blood samples. Other forms, such as point of care testing for hearing loss and congenital cyanotic heart disease and urine screening as is still done in the Canadian province of Quebec are not considered here [5].

3.2 General Aspects of Newborn Screening

3.2.1 Aims and Criteria

The initial aim of newborn screening was to identify infants with serious but treatable disorders, so as to facilitate interventions to prevent or ameliorate the clinical consequences of the disease. .

The classic criteria for screening are those established for the World Health Organisation by Wilson and Jungner in 1968 [6]. They have been revised, extended or condensed repeatedly by regional or national entities, most recently by the American College of Medical Genetics (ACMG) [7]. In reality, the criteria can be simplified and reduced to two main considerations, which would justify screening for any specific disorder: there should be a benefit from neonatal detection, and the overall benefit should be reasonably balanced by the costs of all kinds: the financial costs (opportunity costs) and the risk of harm, if any, to individuals from early detection of the disorder or from false assignment of a positive or negative result. It is important to remember that newborn screening covers the whole process, from sampling to the appropriate referral of an affected baby for the start of treatment and assessment of overall outcome.

Early screening for Phenylketonuria (PKU) due to phenylalanine hydroxylase (PAH) deficiency quickly exposed problems, because the determination of phenylalanine concentrations as the disease marker was found to be imperfect. Transient elevations were observed in healthy neonates and moderate elevations in milder variants of PAH deficiency as well as in cases affected with debilitating and poorly treatable defects in tetrahydrobiopterin synthesis and recycling [8]. Limited specificity has been a consistent issue for any population screening program but in newborn screening the problem of differential diagnosis and the possibility of detecting conditions with either uncertain clinical relevance or absence of effective treatment options became particularly apparent when amino acid and acylcarnitine profiling was introduced into screening programs. The false positive rate particularly for relatively nonspecific markers appeared to increase [9]. Overall, this led to a slow and unequal expansion of screening programs until the American College of Medical Genetics (ACMG) published the Uniform Panel of 29 conditions which every baby should be screened for and an additional 25 conditions that are mostly identified as part of the differential diagnosis in the screening for the primary conditions [7]

3.2.2 Sensitivity, Specificity and Positive Predictive Value

In assessment of screening tests and for understanding of the screening process, some definitions are important:

- *Sensitivity:* The proportion of subjects with the disorder in question detected by the test.
- *Specificity:* The proportion of subjects without the disorder who have a negative test result.
- *False-negative rate:* The percentage of affected subjects not detected by the test.
- *False-positive rate:* The percentage of unaffected subjects with a positive test result.
- *Positive predictive value:* The chance that a positive result actually indicates an affected individual. Similarly, a *negative predictive value* is the chance that a negative result excludes the disorder. These values depend not only on the specificity or sensitivity of the test.

The sensitivity of a test depends to a large extent on chosen cut-off values, and is a balancing act: the higher

the sensitivity the lower the specificity. As the number of conditions under consideration for inclusion in newborn screening programmes continues to increase, and considering that many disease markers are not disease specific, if health care costs are to be contained the issue of screening performance gains in importance [9]. Accordingly, screening programmes must improve specificity without having a negative impact on sensitivity. To this end, in 2005 a Newborn Screening Collaborative Project (http://www.region4genetics.org/msms_data_project/) in the USA began to establish clinically validated cut-off ranges for all conditions identifiable by amino acid and acylcarnitine analysis by MS/MS as well as tools to support multi-analyte result interpretation. At the end of 2010, more than 120 newborn screening programmes worldwide are actively participating in this project. More recently, the Newborn Screening Translational Research Network (www.nbstrn.org) has begun to emulate this project by expanding it to other screened conditions. Another means to improve screening performance is to improve the specificity of the screening tests by including markers with higher disease specificity either in the primary screening test (e.g. succinylacetone for tyrosinemia type I [10] or as second-tier tests (e.g. molecular genetic analysis in cystic fibrosis screening [11]. Second-tier tests are performed on the original newborn screening sample, and their results override those of the primary screen. Although implementation of such assays requires additional resources, for most conditions the second-tier tests could be regionalised by collaborative efforts of screening laboratories, making them cost-effective [12].

3.2.3 Technical Aspects of Newborn Screening Tests

- **Blood-collection-paper Samples**

Newborn screening tests are mainly carried out on blood spots dried on specially manufactured filter paper, usually obtained by heel-stick and necessitating less than 1 ml of blood.

- **Methods**

Following elution of relevant biomarkers from filter paper samples, most modern laboratory technologies can be applied to their quantitation and/or identification. The original bacterial inhibition assays have been abandoned for more accurate, faster and efficient methods that are not prone to interference by the antimicrobial treatment common on neonatal intensive care units, or dependent on sufficient dietary intake of natural protein. With the number of conditions included in newborn screening programmes, the selection of methods, such as MS/MS, that allow determination of multiple analytes in a single blood spot punch is preferred, to minimise the analytical workload. The methodology to some extent varies with the analyte of interest (for example, hormone analyses are usually done by immunoassays), and cost, sensitivity and specificity vary according to the method used. While quantitative results are obtained, the precision of tests using a paper sample is lower than with a plasma or serum sample because of the matrix, collection process, haematocrit variations, etc.

- **Timing of the Test**

The timing of the test also has an important influence on the results. A newborn during the first 72 h is catabolic to some extent, and this is very useful for detecting disorders of intermediary metabolism. There is a paucity of data, except for PKU and CH screening, about results of screening in the first 24 h of life, and this is generally not recommended. Cut-off values adopted to indicate a positive test result for the different analytes typically do not vary with the age of the child at screening; nevertheless, the sensitivity of a test for detecting certain disorders may vary according to age [13], [14].

- **Cut-off Values**

Determination of the cut-off point for each analyte is of necessity a compromise between the aims of perfect sensitivity (detecting all the cases) and keeping the false-positive rate as low as possible. It is important for the laboratory to keep in mind that some analytes fluctuate in the newborn period and all available clinical information that may be captured on the screening card (i.e. gestational age, birth weight, age at first feeding, type of feeding, age at sample collection, transfusion, etc.) should be considered in the evaluation of screening results, which should also be compared with the analyte range that can be expected in true-positive samples [15].

Physicians must bear in mind that no screening test ever gives a perfect performance, although some may come close. If clinical presentation suggests a disorder which is included in newborn screening a test should be done, even if the screening test was negative.

- **Performance**

With the expansion of newborn screening programmes to include more conditions, newborn screening test performance becomes increasingly important [9]. However, data on newborn screening performance are limited, with only a few programmes having published their experiences [16], [17]. ◘ Table 3.1 provides the actual performance metrics for the Minnesota Newborn Screening Program

Table 3.1. Performance metrics of assays performed in the Minnesota Newborn Screening Program between January 2008 and December 2009. Shown also is the (cumulative) impact that test performance has on the average number of cases requiring follow-up per month (based on 100,000 newborns per year)

Condition	FPR	PPV	Detection rate	Unnecessary evaluations/month (/100,000 births per year)
Aminoacidopathies	0.02%	35%	1 : 7457	2
FAO disorders	0.03%	42%	1 : 4722	2
Organic acidurias	0.03%	42%	1 : 4427	3
Biotinidase deficiency	0.08%	10%	1 : 10898	7
CAH (with 2nd tier)	0.08%	9%	1 : 11806	7
CH	0.20%	27%	1 : 1362	16
Cystic fibrosis	0.33%	6%	1 : 5247	28
Galactosaemia	0.06%	21%	1 : 6440	5
Haemoglobinopathies	0.02%	61%	1 : 3014	2
TOTAL	**0.86%**	**20%**	**1 : 463**	**71**

CAH, Congenital adrenal hyperplasia; *CH*, congenital hypothyroidism; *FAO*, fatty acid oxidation; *FPR*, false-positive rate; *PPV*, positive predictive value

and shows what impact performance has on the number of cases that require follow-up for abnormal screening results. As is apparent from these data, screening assays with high false-positive rates and low positive predictive value can result in a significant workload for the follow-up system.

3.2.4 Range of Possibilities from Early Detection

Newborn screening has opened new perspectives in preventive medicine. Babies with disorders of amino acid, organic acid, and fatty acid metabolism are now often detected in the newborn screening laboratory, rather than by the clinical metabolic service. Early detection by newborn screening typically provides three possibilities:

1. The disorder may present in the first days of life, before any newborn screening result is available. Disorders in this category include neonatal presentations of urea cycle defects, organic acidaemias such as methylmalonic acidaemia and, less commonly, almost any of the fatty acid oxidation defects. Detection by newborn screening may not benefit some cases in this category directly. However, it seems appropriate to include these early-presenting disorders in the screening menu, as some may have delayed diagnosis, and on occasion a diagnosis may never be made – the baby having been thought to have died from sepsis.
2. The disorder may be later presenting, and an effective treatment can beneficially alter the natural history. Most conditions included in newborn screening programmes fall into this category.
3. The disorder may be benign, or largely so, and cases will have no benefit from early diagnosis. It is hard to know yet which cases will fit into this category. If that were clear, then the disorders concerned could be removed from the screening menu, but newborn screening, if carefully and sensitively conducted, provides an excellent opportunity for elucidating the natural history of disorders which might or might not fall into this category. What is clear is that mild forms of several disorders will readily be detected by newborn screening, but may not need treatment. One example is short-chain acyl-CoA dehydrogenase (SCAD) deficiency [18].

3.2.5 Follow-up

Once newborn screening results become available, the health care provider must inform the families of the results and initiate follow-up. Table 3.2 lists the biomarkers used in newborn screening for metabolic disorders and their differential diagnosis, and provides recommen-

Table 3.2. Measurable markers included in many newborn screening programmes for inborn errors of metabolism and their differential diagnosis. In addition, information about the initial follow-up tests indicated for confirmation and assessment of the urgency of initiation of follow-up are provided. Also shown are useful secondary markers and marker ratios as well as available second-tier tests. (Expanded/modified from Table D1.2 in [19]

Informative marker	Differential diagnosis			Critical ratio	2nd tier test	Initial confirmatory testing[2]	Urgency of clinical action[3]
	PRIMARY conditions[1]	SECONDARY conditions[1]	OTHER conditions				
Alanine (Ala)	-	-	LA	-	-	*PAA, L&P*	*Low*
Arginine (Arg)	ARG	CIT II	-	-	-	*PAA*	*Moderate*
Argininosuccinic acid (ASA)	ASL	-	-	ASA/Arg	-	*PAA, UOA*	*High*
Citrulline (Cit)	CIT I, ASL	CIT II	PC, OTC, CPS	-	-	*PAA, UOA*	*High*
Glutamic acid (Glu)	-	-	OTC, CPS	Glu/Cit	-	*PAA, UOA*	*Uncertain*
Glutamine + Pyroglutamic acid (Gln+Pyrog)	-	-	OXO-PRO, OTC, CPS	Gln/Cit	-	*PAA, UOA, OROT*	*Uncertain*
Glycine (Gly)	-	-	NKHG	-	-	*PAA, CSF-Gly*	*Low*
Leucine/Isoleucine/OH Proline (Xle)	MSUD	-	PDH (E3), OH-PRO	-	Allo-Ile	*PAA, UOA*	*High*
Lysine (Lys)	-	DE-RED	H-LYS	-	-	*PAA, PAC*	*Low*
Methionine (Met)	CBS	MET	MTHFR, Cbl E, Cbl G, Cbl D v1	Met/Phe	tHcy	*PAA, tHcy*	*High*
Ornithine+Asparagine (Orn+Asn)	-	-	H-ORN, HHH	-	-	*PAA, UAA*	*Uncertain*
Phenylalanine (Phe)	PKU	H-PHE, BIOPT (BS), BIOPT (REG)	Untreated maternal PKU[4]	Phe/Tyr	-	*PAA, UPTR*	*High*
Proline (Pro)	-	-	HPI, HPII, LA	-	-	*PAA*	*Low*
Succinylacetone (SUAC)	TYR I	-	-	-	-	*PAA, UOA, AFP*	*Moderate*
Threonine (Thr)	-	CIT II	-	-	-	*PAA*	*Low*
Tyrosine (Tyr)	TYR I	TYR II, TYR III	-	-	SUAC	*PAA, UOA*	*Moderate*
Valine (Val)	MSUD	-	PDH (E3), VAL	-	Allo-Ile	*PAA, UOA*	*High*
Carnitine (C0)	CUD	CPT I	Untreated maternal cases (CUD, GA I, 3MCC)[D]	C0/(C16+C18)	-	*PCarn, PAC*	*Moderate*
Propionylcarnitine (C3)	PA, MCD, MUT, Cbl A/B	Cbl C/D	SUCLA2, untreated maternal Vit B12 def[4]	C3/C2	MMA, MCA, tHcy	*PCarn, PAC, UOA*	*High*
Formiminoglutamic acid (FIGLU)	-	SCAD, IBG	FIGLUaciduria	-	-	*PAC, UOA*	*Low*
Butyryl-/isobutyrylcarnitine (C4)	-	SCAD, IBG, GA II	EE, FIGLUaciduria	-	EMA	*UAG, UOA, PAC, UAC*	*Low*
Tiglylcarnitine (C5:1)	BKT	2M3HBA	-	-	-	*See C5-OH*	*High*
Isovaleryl-/2-methylbutyrylcarnitine (C5)	IVA	2MBG, GA II	EE	-	-	*UAG, UOA, PAC*	*High*
OH Butyrylcarnitine (C4-OH)	BKT	2M3HBA, S/MCHAD	-	-	-	*UOA, PAC*	*Uncertain*

Table 3.2. *Continued*

Informative marker	Differential diagnosis			Critical ratio	2nd tier test	Initial confirmatory testing[2]	Urgency of clinical action[3]
	PRIMARY conditions[1]	SECONDARY conditions[1]	OTHER conditions				
Hexanoylcarnitine (C6)	MCAD	GA II	-	-	-	*See C8*	
OH Isovalerylcarnitine (C5-OH)	3MCC, HMG, BKT, MCD	2M3HBA, 3MGA	BIOT (partial)	C5-OH/C0, C5-OH/C8	-	*UOA, PAC, UAC*	*Low for 3MCC, High for other conditions*
Octanoylcarnitine (C8)	MCAD	GA II, MCKAT	-	C8/C2	-	*UAG, UOA, PAC*	*High*
Malonyl-/OH Octanoylcarnitine (C3DC)	-	MAL, MCKAT	-	C3DC/C10	-	*UOA, PAC*	*High*
Decadienoylcarnitine (C10:2)	-	DE-RED	-	-	-	*PAC, PAA*	*Uncertain*
Decenoylcarnitine (C10:1)	MCAD	GA II	-	-	-	*See C8*	*See C8*
Decanoylcarnitine (C10)	MCAD	GA II	-	-	-	*See C8*	*See C8*
Succinyl-/methylmalonylcarnitine (C4DC)	MUT, Cbl A,B	-	SUCLA2	-	MMA, MCA, tHcy	*UOA, PAC*	*Low*
Glutaryl-/OH Decanoylcarnitine (C5DC)	GA I	GA II	-	C5DC/C5OH	-	*UOA, PAC, UAC*	*High*
Dodecenoylcarnitine (C12:1)	VLCAD	-	-	-	-	*See C14:1*	
Dodecanoylcarnitine (C12)	VLCAD	-	-	-	-	*See C14:2*	
Methylglutarylcarnitine (C6DC)	HMG	-	-	-	-	*See C5-OH*	
Tetradecanedioylcarnitine (C14:2)	VLCAD, LCHAD/TFP	GA II	-	-	-	*See C14:1*	
Tetradecenoylcarnitine (C14:1)	VLCAD, LCHAD/TFP	GA II	-	C14:1/C2, C14:1/C16	-	*PAC, UOA, DANN*	*High*
Tetradecanoylcarnitine (C14)	VLCAD, LCHAD/TFP	CACT, CPT II	-	-	-	*See C14:1*	
Palmitoylcarnitine (C16)	VLCAD	CPT I (low), CACT, CPT II	-	-	-	*PCarn, PAC, UOA*	*High*
OH Hexadecenoylcarnitine (C16:1-OH)	LCHAD/TFP	-	-	-	-	*See C16-OH*	
OH Palmitoylcarnitine (C16-OH)	LCHAD/TFP	-	-	C16-OH/C16	-	*PCarn, PAC, UOA*	*High*
Linoleylcarnitine (C18:2)	LCHAD/TFP	CPT II	-	-	-	*See C16*	
Oleylcarnitine (C18:1)	VLCAD, LCHAD/TFP	CPT I (low), CACT, CPT II, GA II	-	-	-	*See C16*	
Stearylcarnitine (C18)	VLCAD	CPT I (low), CACT, CPT II, GA II	-	-	-	*See C16*	

Table 3.2. *Continued*

Informative marker	Differential diagnosis			Critical ratio	2nd tier test	Initial confirmatory testing[2]	Urgency of clinical action[3]
	PRIMARY conditions[1]	SECONDARY conditions[1]	OTHER conditions				
OH Oleylcarnitine (C18:1-OH)	LCHAD/TFP	-	-	-	-	*See C16-OH*	
OH Stearylcarnitine (C18-OH)	LCHAD/TFP	-	-	-	-	*See C16-OH*	
Biotinidase (BIOT)	BIOT	-	-	-	-	*Biotinidase in serum*	*Moderate*
Galactose-1-phoshate uridyltransferase (GALT)	GALT	-	-	-	-	*GALT in RBC*	*High*
Total galactose	GALT	GALK, GALE	-	-	-	*GALT in RBC, Gal-1-P, GALK/ GALE in RBC*	*High*
Glucose-6-phosphate dehydrogenase (G6PD)	G6PD	-	-	-	DNA	*G6PD in RBC*	*High*
Galactocerebrosidase (GALC)	Krabbe disease	-	-	-	DNA	*GALC in WBC, DNA*	*High*
Acid alpha-glucosidase (GAA)	Pompe disease	-	0	-	DNA	*GAA in WBC, DNA*	*High*
Alpha-galactosidase A (GLA)	Fabry disease	-	Obligate carrier mother	-	DNA	*GLA in WBC, DNA*	*Low*

AC, Sum of selected species (C0+C2+C3+C16+C18+C18:1); *AFP*, alpha fetoprotein; ; *Allo-Ile*, allo-isoleucine; *ARG*, arginase deficiency; *ASL*, argininosuccinate lyase deficiency; *BIOPT (BS)*, GTP cyclohydrolase and 6-pyruvoil tetrahydropterin synthase deficiencies; *BIOPT (REG)*, dihydropteridine reductase and pterin-4α-carbinolamine dehydratase 2 deficiencies; *BIOT*, biotinidase deficiency; *BKT*, beta-ketothiolase deficiency; *CACT*, carnitine-acylcarnitine translocase deficiency; *CBL*, cobalamin metabolism defect, complementation type A/B/C/D variant 1/E or F; *CBS*, cystathionine beta-synthase deficiency; *CIT II*, citrullinemia type II (citrin deficiency); *CPS*, carbamoyl phophaste synthetase type I deficiency; *CPT I*, carnitine palmitoyltransferase type I deficiency; *CPT II*, carnitine palmitoyltransferase type II deficiency; *CSF-Gly*, glycine in cerebrospinal fluid; *CUD*, carnitine uptake defect; *DE-RED*, 2,4-dienoyl-CoA reductase deficiency; *DNA*, molecular genetic analysis ; *EE*, ethylmalonic encephalopathy; *EMA*, ethylmalonic acid; *GA I*, glutaric aciduria type I; *GA II*, glutaric aciduria type II; *Gal-1-P*, galactose-1-phosphate in red blood cells ; *GALE*, UDP-galactose-4-epimerase deficiency; *GALK*, galactokinase deficiency; *GALT*, galactose-1-phosphate uridyltransferase deficiency; *G6PD*, glucose-6-phosphate dehydrogenase deficiency; *HHH*, hyperornithinaemia-hyperammonaemia-homocitrullinuria syndrome; *H-LYS*, alpha-aminoadipic semialdehyde synthase deficiency; *HMG*, 3-hydroxy methyl-glutaryl-CoA synthase deficiency; *H-ORN*, hyperornithinaemia; *H-PHE*, mild phenylalanine hydroxylase deficiency; *HPI*, hyperprolinaemia type I (proline oxidase deficiency); *HPII*, hyperprolinaemia type II (pyrroline-5-carboxylate dehydrogenase deficiency); *IBG*, isobutyryl-CoA dehydrogenase deficiency; *IVA*, isovaleric acidaemia; *LA*, lactic acidaemia (primary or secondary); *LCHAD/TFP*, long-chain 3-hydroxy acyl-CoA dehydrogenase deficiency, trifunctional protein deficiency; *L&P*, plasma lactate and pyruvate ; *MAL*, malonyl-CoA decarboxylase deficiency; *2MBG*, 2-methylbutyrylglycinuria; *MCA*, methylcitric acid; *MCAD*, medium-chain acyl-CoA dehydrogenase deficiency; *3MCC*, 3-methylcrotonyl-CoA carboxylase deficiency; *MCD*, multiple carboxylase deficiency; *MCKAT*, medium-chain 3-ketoacyl-CoA thiolase deficiency; *MET*, methionine adenosyltransferase I/III, *S*-adenosylhomocysteine hydrolase, and glycine *N*-methyltransferase deficiencies; *3MGA*, 3-methylglutaconic aciduria type I; *2M3HBA*, 2-methyl 3-hydroxybutyric aciduria; *MMA*, methylmalonic acid; *MSUD*, maple syrup urine disease; *MTHFR*, methylene tetrahydrofolate reductase deficiency; *MUT*, methylmalonyl-CoA mutase deficiency; *NKHG*, nonketotic hyperglycinaemia; *OAT*, ornithine aminotransferase deficiency; *OH-PRO*, hydroxyprolinaemia (hydroxy-l-proline oxidase deficiency); *OROT*, urine orotic acid; *OTC*, ornithine transcarbamoylase deficiency; *OXO-PRO*, 5-oxoprolinaemia (glutathione synthetase deficiency); *PAA*, plasma amino acids ; *PAC*, plasma acylcarnitines; *PC*, pyruvate carboxylase deficiency; *PCarn*, plasma carnitine ; *PDH (E3)*, pyruvate dehydrogenase component E3 deficiency (dihydrolipoamide dehydrogenase deficiency); *PKU*, phenylalanine hydroxylase deficiency; *RBC*, red blood cells; *SCAD*, short-chain acyl-CoA dehydrogenase deficiency; *S/MCHAD*, short-/medium-chain acyl-CoA dehydrogenase deficiency; *SUCLA2*, succinyl-CoA synthase deficiency; *tHcy*, total homocysteine ; *TYR I*, tyrosinaemia type I (fumarylacetoacetase deficiency); *TYR II*, tyrosinaemia type II (tyrosine aminotransferase deficiency); *TYR III*, tyrosinaemia type III (4-hydroxyphenylpyruvate dioxygenase deficiency); *UAA*, urine amino acids;*UAC*, urine acylcarnitines; *UAG*, urine acylglycines; *UOA*, urine organic acids; *UPTR*, urine pteridines; *VAL*, valine transaminase deficiency; *VLCAD*, very long-chain acyl-CoA dehydrogenase deficiency; *WBC*, white blood cells.

[1]Primary and secondary conditions based on current recommendations by the US Secretary of Health and Human Services. [2]Tests performed in other than dried blood spot samples; may include additional tests when newborn is clinically symptomatic. [3]Further information on follow up requirements can be found at: http://www.acmg.net/resources/policies/ACT/condition-analyte-links.htm. [4]Detection is possible because of secondary carnitine deficiency of the newborn

dations for follow-up investigations and the urgency of follow-up initiation. To facilitate a timely and appropriate response to abnormal results, most newborn screening programmes have developed follow-up protocols. As an example the American College of Medical Genetics (ACMG) made available oneline (http://www.acmg.net) clinical descriptions and recommendations for clinical and laboratory follow-up for each condition detectable by newborn screening.

3.3 Screening for Individual Inborn Errors of Metabolism

Over 50 inborn errors of metabolism can now be detected by newborn screening, with varying degrees of certainty. This section will concentrate on inborn errors that are usually referred to metabolic physicians. ◘ Table 3.2 shows the markers detectable by newborn screening programmes, applicable secondary markers and ratios, and the primary follow-up tests indicated.

3.3.1 Aminoacidopathies

▪ Phenylketonuria (PKU)

▪▪ Test Methods

Screening for PKU has been conducted in most developed countries since the 1960s. The initial test was the 'Guthrie test', a bacterial inhibition assay. While this assay is still used in a few screening laboratories, most have replaced it with alternative methods such as fluorometry, calorimetry and, more recently, MS/MS.

▪▪ Timing

Screening in the United States usually takes place after 24 h, but elsewhere 48 h plus is typical. As with other amino acids, levels of phenylalanine rise steadily during the 1st days of life, and there is a theoretical risk of missing PKU if the test is conducted too early. However, testing by MS/MS and measuring the ratio of phenylalanine to tyrosine overcomes this problem [20].

▪▪ Reliability

The bacterial inhibition assay was a robust test, and when applied to samples taken at 48 h or more, any false-negative test results in subjects with classic PKU have almost always been due to errors of process (sample not taken, clerical error, etc.) rather than biological variation or problems with the actual test procedure [21]. In addition, treatment with antibiotics prior to sample collection can cause negative results [22]. MS/MS screening is very reliable owing to its sensitivity and its ability to simultaneously measure other amino acids, allowing determination of the phenylalanine-to-tyrosine ratio in every sample [20]. Overall, the sensitivity of all PKU tests is very high and, with experience, the specificity is also high. There are no established benchmarks.

▪▪ Outcome

Patients obtaining good control of phenylalanine levels by 3-4 weeks and maintaining good average control have a good neuropsychological outcome. There are still minor deficits, and *maternal PKU* remains a potential problem. These aspects are reviewed in ► Chapter 17.

▪ Disorders of the Urea Cycle

Citrullinaemia and *argininosuccinic aciduria*, either severe or later presenting, can be diagnosed with apparently high sensitivity by measuring citrulline, and also argininosuccinic acid in the latter. There are problems because of the recent description of mild, asymptomatic citrullinaemia. Detection of severe, early-presenting *argininaemia* by measuring arginine has also been described. *Carbamoyl phosphate synthetase* and *ornithine transcarbamoylase (OTC) deficiencies* cannot be so easily detected. Low citrulline is an indicator, but a low cut-off for citrulline overlaps with low citrulline seen in sick neonates in general. Diagnosis of OTC deficiency has been described by the detection of pyroglutamic acid, derived from glutamine, and a blood-spot method for detecting glutamine has been described [23]. To date newborn screening is quite unreliable for these early disorders but the clinical validation of additional markers may overcome this obstacle. A related disorder, *citrin deficiency* causing neonatal hepatitis, (citrullinaemia type II) could be detected by a disturbance of several amino acids, especially moderately elevated citrulline, and detection of several cases by MS/MS has been reported. If ornithine is one of the analytes included, *hyperornithinaemia, hyperammonaemia, homocitrullinuria (HHH syndrome)* and *ornithine aminotransferase deficiency* could theoretically be detected, but no cases have been reported, probably because these conditions do not cause elevated ornithine levels in early infancy [24].

▪ Other Aminoacidopathies

Maple syrup urine disease, the second aminoacidopathy added to newborn screening programmes after phenylketonuria, was initially detected by chromatography, then bacterial inhibition assay of leucine and perhaps valine. Since MS/MS detection as applied in newborn screening cannot distinguish among isotopes, the leucine peak encompasses isoleucine, allo-isoleucine and hydroxyproline. Classic MSUD can readily be detected

(although a positive result could also indicate benign hyperhydroxyprolinaemia) but not all variant cases can be distinguished, even by the application of second-tier assays for allo-isoleucine [25]. A result indicating classic MSUD needs to be handled as an emergency. *Cystathionine synthase deficiency (homocystinuria)* is detected by an elevated methionine level and a high methionine/phenylalanine ratio confirmed by application of second-tier molecular and biochemical methods that are becoming increasingly available [26]. It appears that current screening approaches cannot however detected pyridoxine-responsive homocystinuria; detection of a truly responsive case by newborn screening has not been reported. The *tyrosinaemias* still present a problem in most screening programmes. In *tyrosinaemia type 1* (*fumaryl acetoacetase deficiency*) the blood tyrosine level in newborns often overlaps with those observed in the unaffected population. Several cases of tyrosinaemia type 1 have been reported as missed by MS/MS screening, but as it is now possible to include succinylacetone in the analysis of amino acids and acylcarnitines by MS/MS, this situation should improve significantly [10]. *Tyrosinaemia type II* is readily detectable, and at least one case of tyrosinaemia type III has been detected prospectively by newborn screening.

Several other amino acids can be measured simultaneously by MS/MS without altering the method, including alanine, arginine, glutamine, glutamic acid, glycine, ornithine and serine. Arginine and argininosuccinic acid are valuable in the identification of cases with arginase and argininosuccinic lyase deficiencies. Glycine was expected to be elevated in cases of nonketotic hyperglycinaemia, however, there proved to be significant overlap of glycine levels between the unaffected population and affected cases, and it is this is typically not used for screening [27]. Similarly, alanine is not sufficiently informative for the detection of primary lactic acidaemias, but is useful for the detection of other conditions when part of analyte ratios (http://www.region4genetics.org/msms_data_project/).

3.3.2 Galactosaemias

Galactose-1-phosphate uridyl transferase (GALT) deficiency, galactokinase deficiency and galactose epimerase deficiency can all be detected by newborn screening.

- **Test Methods**

Methods used in screening are measures of metabolites, galactose and galactose-1-phosphate, or measures of enzyme activity, confined to the Beutler test for GALT. Most commonly now, a metabolite assay is followed by confirmatory testing using a GALT assay and quantitative determination of galactose and galactose-1-phosphate.

- **Reliability**

A combination of these methods will provide a precise diagnosis of transferase deficiency (but see below), and an indication of galactokinase deficiency, in which there is elevation of galactose alone and a normal GALT activity. However, the differentiation of red-cell epimerase deficiency (a benign condition) and systemic epimerase deficiency, which is clinically similar to transferase deficiency, may not be clear. Additionally, moderate metabolite elevations and severely reduced, but not absent, GALT activity are seen in combined heterozygosity for a severe mutation in the transferase *GALT* gene, and a common 'Duarte' mutation. The differentiation is important, as a Duarte/galactosaemia double heterozygote does not need treatment [28].

- **Outcome**

Despite early identification and treatment, the long-term outcome for transferase deficiency remains a challenge, with about half the children having early intellectual problems, and some evidence of ongoing deterioration in most (► Chapter 7). There is no evidence that presymptomatic treatment alters outcome (although death may be avoided in a few), and because of this, not all developed countries screen for the galactosaemias. Treated galactokinase deficiency would be expected to have a good outcome, but is much rarer than transferase deficiency, and systemic epimerase deficiency is rarer still, with little known of the long-term effects of screening for it.

3.3.3 Organic Acid Disorders

Organic acids that form acylcarnitines can be detected by MS/MS, and a large number of organic acid disorders have been so detected (■ Table 3.2). The classic organic acid disorders, *methylmalonic* (MMA), *propionic* (PA) and *isovaleric* (IVA) *acidaemias* can readily be detected, although the baby may be symptomatic before newborn screening results are available. An elevation of propionylcarnitine (C_3) might indicate either PA, MMA, vitamin B_{12} deficiency secondary to maternal deficiency or *cobalamin C defect* (methylmalonic aciduria with homocystinuria), or possibly *cobalamin D* or *F defects*. While severe neonatal-onset MMA will involve elevated C_3 levels, other defects can be more reliably detected by using the ratio of C_3 to acetylcarnitine and second-tier tests for MMA and methylcitric acid [26]. Special impor-

tance attaches to *glutaric acidaemia type I* (glutaryl CoA dehydrogenase deficiency). The marker compound, glutarylcarnitine (C_5DC), which is also one of several markers for *glutaric acidaemia type II*, may be only marginally elevated, especially if the infant is sampled after the 1st week. Again, the application of analyte ratios, as with 3-hydroxy isovaleryl-, octanoyl- and palmitoylcarnitine, is more discriminatory (http://www.region4genetics.org/msms_data_project/).

Newborn screening has uncovered an unexpectedly high frequency of cases of *3-methylcrotonyl CoA carboxylase deficiency* (MCCC), which was previously thought to be a relatively rare finding, and asymptomatic cases of maternal MCCC are also detected regularly by newborn screening [29]. This disorder is one of several that might be benign in most instances. Other maternal organic acid disorders uncovered by abnormal results on neonatal screening include milder variants of holocarboxylase synthase deficiency, glutaric acidaemia type I and vitamin B_{12} deficiency.

- **Biotinidase Deficiency**

A specific enzyme assay on dried blood spots is used to detect biotinidase deficiency because MS/MS based metabolite testing fails to identify most cases.

3.3.4 Fatty Acid Oxidation Disorders

Disorders of carnitine uptake, the carnitine cycle, and mitochondrial beta-oxidation can be detected by MS/MS testing of acylcarnitines (◘ Table 3.2). For several disorders, newborn screening programmes have detected more cases than have historically presented clinically [16], [30]. While some of these subjects might never have experienced episodes of decompensation, it is not possible at present to distinguish who is at most risk, and all have by definition a functional defect in oxidation rates. This is especially true of *medium-chain acyl-CoA dehydrogenase (MCAD)* deficiency, the most frequently occurring fatty acid oxidation disorder, in which the detection rate is nearly doubled. MCAD deficiency is reliably indicated by elevated octanoylcarnitine (C_8). The ratios of C_8 to C_{10} (decanoylcarnitine) and C_8 to C_2 (acetylcarnitine) are also commonly used as secondary markers. Some laboratories employ a second-tier molecular genetic assay to determine the presence of at least the common Northern European mutation, c.985A>G. However, the allele frequency of this mutation is lower (often less than 70%) in screen-detected than in clinically presenting patients, and some other mutations found by screening have not been recorded in clinical presentations [31], [32]. Elevated urinary acylglycines and abnormal plasma acylcarnitine profiles virtually confirm the diagnosis of MCAD deficiency. More definitive confirmation can be obtained by enzymatic or DNA analysis or more simply by acylcarnitine profiling in cultured skin fibroblasts. Newborn screening for *very-long-chain acyl-CoA dehydrogenase (VLCAD)* deficiency has revealed a problem in the traditional request for just a repeat dried blood spot submission when an abnormal screening result has been obtained. For some conditions which only become clinically manifest in response to catabolic stress, collecting a repeat sample when the infant is metabolically stable following the stress of the perinatal period can be be associated with a false negative result. This is particularly so for VLCAD deficieny [33]. Accordingly, follow-up of an abnormal result should routinely include more specific testing, often molecular genetic analysis or specific enzyme assay. *Short-chain acyl-CoA dehydrogenase (SCAD)* deficiency is found more commonly in screened patients than would have been expected from previous experience, and all cases reported so far have been asymptomatic. As mentioned for some organic acid disorders, newborn screening for fatty acid oxidation disorders has also led to the identification of a milder variant of *carnitine uptake deficiency (CUD)* in mothers whose carrier offspring have abnormally low free carnitine concentrations in their screening samples [34]. Other fatty acid oxidation disorders found by newborn screening are shown in ◘ Table 3.2.

3.3.5 Lysosomal Storage Disorders

Screening for lyosomal storage disorders has raised several problems, not least because many of the disorders have quite a late onset. Krabbe disease (galactocerebrosidase deficiency) however, usually presents early, and requires rapid treatment for any hope of success [35]. Krabbe disease was introduced to the New York State Newborn Screening Program in 2006 [36], with the goal of identifying patients who can benefit from early bone marrow transplantation. However, even with a screening strategy that combines a specific enzyme assay with second-tier DNA sequencing of the *GALC* gene, the resources required to follow up on abnormal results are extensive, yet still not always conclusive, leaving families with uncertainty of what to expect of the long-term outcome of their children with low enzyme activity and at least one mutation identified. Nevertheless, addition of four other lysosomal storage disorders (Pompe, Gaucher, Fabry and Niemann-Pick A/B diseases) has already been mandated in a few US states (Illinois, Missouri and New Mexico),

and screening for Pompe and Fabry diseases is active in Taiwan [36], [37]. Screening for Fabry disease adds new complexity to follow-up activities, because most infants identified are affected with the later onset variant of the disease and at minimum their obligate carrier mothers require clinical evaluation, genetic counselling and, potentially, treatment. Finally, the cost of enzyme replacement for these disorders and the potentially difficult provision of a consistent supply of recombinant enzyme are further issues that follow-up health care providers and affected families will have to face [38] .

3.3.6 Other Conditions

Other disorders often tested for in various combinations in newborn screening programmes, but of more importance for general paediatricians or those with other specialties, include the following (◘ Table 3.2), described here briefly.

- **Congenital Hypothyroidism (CH)**

Screening for primary CH is universal in countries with well-developed health systems, and widely practised in less well-developed countries. Testing is done by measurement of thyroid stimulating hormone (TSH) or, increasingly less commonly, of thyroxine (T4) with TSH as a second-tier test. Trial withdrawal of treatment at 2-3 years will identify cases that were transient and do not need life-long treatment.

- **Congenital Adrenal Hyperplasia (CAH)**

Measurement of 17-hydroxyprogesterone by immunoassay is the primary test for CAH. The test readily identifies cases, but the false-positive rate is high, especially among preterm babies (up to 1% of all newborns, and a much greater proportion of premature babies). A cost-effective approach to elevated 17-hydroxyprogesterone levels is to perform either a primary or a second-tier test by liquid chromatography (LC) MS/MS for the determination of 17-OHP and other steroids in the cortisol biosynthesis pathway. This approach can eliminate more than 90% of potential false-positive results of the primary screening [12].

- **Cystic Fibrosis**

Screening and early diagnosis have produced clear-cut nutritional benefit and improvement in lung function. The test involves measurement of immunoreactive trypsin (trypsinogen) and usually second-tier molecular genetic testing for some CFTR mutations. Some screening programmes have been functioning for almost over 30 years, and screening is widely recommended.

- **Glucose-6-phosphate Dehydrogenase Deficiency**

An NAD/NADH-based enzyme assay can detect this disorder. The test is widely used in Asian countries, while countries with populations of primarily caucasian origin have so far been reluctant to include this condition into their screening programmes. The usefulness in avoiding kernicterus and haemolytic crises is likely, but has not been well demonstrated.

- **Haemoglobinopathies**

Sickle-cell disease and other hemoglobinopathies are included in areas where this is prevalent, but are now also included in all screening programmes in the USA following recommendations by the Secretary of Health.

- **Severe Combined Immunodeficiency Syndrome (SCID)**

Severe combined immunodeficiency syndromes can be detected by the application of DNA-based technologies to identify T-cell lymphopenia in affected infants, using dried blood spot samples. This was first undertaken in Wisconsin, USA, as of 2008 [39] and became a recommended screen for the USA in 2010 (http://www.hrsa.gov/heritabledisorderscommittee/uniformscreeningpanel.htm). Additional states and countries are now beginning to also include this group of conditions into their screening programs.

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Diagnostic Procedures: Functional Tests and Post-mortem Protocol

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4.1 Introduction

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. If an inherited metabolic disease is suspected then blood, urine and cerebrospinal fluid should be collected for the appropriate investigations (► Chapter 1, Table 1.3). 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.

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.

4.2 Basal Investigation

4.2.1 Amino and Organic acids Analyses

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

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 immediately performed. In non acute situations, a 24-h or 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 mild homocystine elevation 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 as compared with the laboratory's age-related reference values and relative to other constituents. In many cases, the final interpretation needs knowledge both of the clinical and nutritional status and of the conditions of sampling, and 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 4.1. The main abnormal organic acids that may be found in IEM are given in ◘ Table 4.2.

4.2.2 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 4.3. To allow reliable interpretation of the results, the correct method of sampling and processing blood and urine is specified in ◘ Table 4.4. It is important to record the conditions under which sampling is undertaken; for example, either acute or chronic 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 now 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].

Table 4.1. Interpretation of the principal variations in plasma amino acids

Plasma amino acid	Variation from normal	Other plasma amino acids	Investigations in other fluids	Diagnoses
Alanine	↑	See Gln, Pro, Gly		Hyperlactacidaemia
Arginine	↑	Gln ±↑, Cit ±↓, Orn↓	U: ±↑	Arginase def
	↓	Gln ±↑, Pro↓, Cit↓, Orn ±↓ Orn↓, Lys↓	U: ↑++, UOA: orotic↑	P5CS def LPI
Arginino-succinic acid	±↑	Gln ±↑, Cit ±↑	U: ASA↑	ASLD Late-onset form
	↑	Gln↑, Cit↑	U: ASA↑++	ASLD neonatal form
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 def
	↓	Met↑, Tyr↑		Hepatic failure
		Cit↑, Cys 2↑, 3Mhis↑		Renal failure
Citrulline	↓ or ↑	See Gln		UCDs
	↑	Cys2↑, 3MHis↑, BCAA↓ Alone ±↑		Renal failure Heterozygote ASS
Cys2	↓+++	SulfoCys↑	U:SulfoCys↑, Tau↑	Sulfite oxidase def
	↑	Cit↑, BCAA↓, 3MHis		Renal failure
	N	All normal	U: Cys2↑, Lys↑, Orn↑, Arg↑	Cystinuria
Glutamine	↑	Cit ↓, Orn ↓, Arg ↓	UOA: Orotic↑ (OTC, OAT)	Mitochondrial UCD, neonatal OAT
		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 ↑, Homocitrulline↑	HHH
		Cit↑, Orn↓, Lys↓,Arg↓	U: Cit↑, Orn↑, Lys↑, Arg↑	LPI
	N or ↓	Cit↑, Ala↑, Lys↑		PC def
		Cit↑, Thr↑, Orn↑, Lys↑, Arg↑		Citrin def
		Cit N, Gly↑, Ala↑, Lys↑		Organic acidurias
	↓ +++		CSF: ↓ +++	Glutamine synthesis def
Glycine	↑	Alone	CSF↑, U↑	NKH
		Ala ↑	CSF N, U↑	Valproate
		Gln N, Ala↑, Lys↑	CSF N, U↑, UOA	Organic acidurias
		Thr↑±	CSF: Gly ↑±, Thr ↑±	PNPO def
Histidine	↑	Alone	U ±↑	Histidase def
Homocystine	±↑	All normal		Secondary
	↑	See Methionine		

Table 4.1. *Continued*

Plasma amino acid	Variation from normal	Other plasma amino acids	Investigations in other fluids	Diagnoses
Lysine	↑++	Alone		Saccharopine dehydrogenase def
	↑ ↑	Gln↑, NH3↑		Urea Cycle Disorders
		Gln↓ , NH3↑, Cit↑±		PC def
	↓	Gln↑, Cit ±↑, Orn↓, Arg↓	U: Cit↑, Orn↑, Lys↑, Arg↑ UOA: Orotic↑	LPI
Methionine	↓	HCy2↑	U: HCy2↑ alone	MTHFR def
			UOA: MMA↑	Cobalamin metabolism
	↑	HCy2↑, Cys2↓, Disulfide	U: HCy2↑	CBS def
		Tyr↑, BCAA↓		Hepatic failure
Ornithine	↑	Alone	U ±↑	OAT def
	↑	Gln↑, Cit↓, HomoCit ±↑	U: Orn ±↑, HomoCit↑	HHH syndrome
	↓	Gln±↑, Pro↓, Cit↓, Arg↓ Arg↓, Lys↓	U: Cit↑, Orn↑ Lys↑, Arg↑ UOA: Orotic↑	P5CS def LPI
Phenylalanine	↑+++	Tyr↓		PKU
	↑±	Tyr ±↓		Biopterin synthesis def
Pipecolic	↑	All normal	CSF: Pip ±↑, P: VLCFA CSF: Pip ±↑, P and U: αAASA	Peroxisomal diseases αAASADH def (B6 responsive seizures)
Proline	↑	Alone	U: N or ↑	Hyperprolinaemia I
		Alone	U: N or ↑, P5C↑	Hyperprolinaemia II
		Ala↑		Hyperlactacidaemia
	↓	Cit↓, Orn↓, Arg↓		P5CS def
Serine	↓	All others N	CSF↓	Serine synthesis def
Sulfocysteine	↑	Cys2 low Cys2 very low	U: SulfoCys 0, all AA normal U: SulfoCys↑, Tau↑	Anticoagulant Sulfite oxidase def
Tyrosine	↑+++	Alone	U: ↑ alone	Tyrosinaemia type II
	↑	Met↑, BCAA↓	UOA: succinylacetone 0	Hepatic failure
			UOA: succinylacetone +	Tyrosinaemia type I

def, Deficiency: Compounds: *AA,* Amino acids; *αAASA*, alpha-aminoadipic semialdehyde; *Ala*, alanine; *alle*, alloisoleucine; *Arg*, arginine; *ASA*, argininosuccinic acid; *BCAA*, branched chain amino acids (valine, isoleucine, alloisoleucine, leucine); *Cit*, citrulline; *Cys2*, cystine; *Disulfide*, disulfide cysteine-homocysteine; *Gln*, glutamine; *Gly*, glycine; *Hcy2*, homocystine; *HomoCit*, homocitrulline; *2KG*, 2-ketoglutarate; *Lac*, lactate; *Lys*, lysine; *Met*, methionine; *3MHis*, 3-methylhistidine; *MMA*, methylmalonic acid; *NH3*, ammonia; *Orn*, ornithine; *P5C*, Δ1-pyrroline-5-carboxylate; *Pip*, pipecolic; *PNPO*, pyridox(am)ine-5-phosphate oxidase; *Pro*, proline; *SulfoCys*, sulfocysteine; *Tau*, taurine; *Thr*, threonine; *Tyr*, tyrosine.
Fluids: *CSF*, cerebrospinal fluid; *U*, urine; *UOA*, urine organic acids; *VLCFA*, very long chain fatty acids.
Diagnoses: *αAASADH*, alpha Aminoadipic semialdehyde dehydrogenase; *ARGD*, arginase deficiency; *ASLD*, argininosuccinic lyase deficiency; *ASSD*, argininosuccinic synthetase deficiency;*CBS*, cystathionine-β-synthase; *HHH,* triple H syndrome (hyperammonaemia, hyperornithinaemia, homocitrullinuria; *LPI,* lysinuric protein intolerance; *MSUD*, maple syrup urine disease; *MTHFR*, methylene tetrahydrofolate reductase; *NKH*, non-ketotic hyperglycinaemia; *OAT*, ornithine aminotransferase; *PC*, pyruvate carboxylase; *P5CS*, Δ1-pyrroline-5-carboxylate synthase; *PKU* phenylketonuria; *UCD*, urea cycle disorder

Table 4.2. Interpretation of urine organic acids analysis

Principal OA	Other OAs	Causes of variation	Other investigations
Adipic	Very high isolated	Nonmetabolic (plastifier?)	
	► Lines: 3-OH- butyric and EMA Remark:		
	If Adipic > Sebacic	Ketosis, β-ox	
	If Adipic < Sebacic	MCT supplementation	
Dicarboxylic acids (DCA)	► Adipic, 3-OH-*n*-butyric and EMA		
3,6-Epoxyoctanedioic	Other epoxy (C10, C12, C12), 2-OH-sebacic, DCA with Adipic>Suberic	Peroxisomal diseases	
	Idem but Adipic<Suberic	MCT supplementation	
Ethylmalonic	>20 µmol/mmol C alone	SCAD	Acylcarnitines
	>20 µmol/mmol C ± IBG, 2MBG, IVG	Valproate, RC, GAII	
	>100 µmol/mmol C + IBG, nBG, 2MBG, IVG, HG, SG, 2OHG, DCA, Glut	ETHE1	
	>100 µmol/mmol C + nBG	GAII	
		SCAD	
Fumaric	High succinate, malate	Fumarase def	
	±High with other KC derivatives+lactate	RC	
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	
Glyceric	D-Isomer	Glycerate kinase deficiency	
	L Isomer	D-glycerate dehydrogenase or glyoxylate reductase def (hyperoxaluria typeII)	
Glycolic	Oxalic	Type I oxalosis	
	4HB	SSADH def	
	Lactic, ethyleneglycol	Ethyleneglycol intoxication	
Hexanoylglycine	High± and SG±	Mild or asymptomatic MCAD def	Acylcarnitines
	High+SG+DCA	MCAD def	
	High + SG + DCA + EMA + Glut + IBG + EMBG + IVG + nBG	GA II	
Homogentisic	Alone	Alkaptonuria	
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-Hydroxybutyric	Alone	Drug addiction	
	4,5 diOH-hexanoic lactone and acid, 3,4-diOH-butyric, 2,4-diOH-butyric, glycolic	Succinic semialdehyde dehydrogenase deficiency	

Table 4.2. *Continued*

Principal OA	Other OAs	Causes of variation	Other investigations
3-Hydroxy-dicarboxylic acids (3HDC)	► 3-Hydroxy-*n*-butyric line		
2-Hydroxy-glutaric	Very high	D- or L-2-OH glutaric aciduria	Acylcarnitines
	High±acylglycines	Glutaric aciduria type II	
	Moderately high	Respiratory chain	
3-Hydroxy-glutaric	Glutaric normal or high	Glutaric aciduria type I	
	3-OH-butyric elevated	Ketosis	
3-Hydroxy-isobutyric	2-Ethylhydracrylic	3OH-isobutyric dehydrogenase deficiency	
2-Hydroxy-isovaleric	2-OH-3-CH3Val, 2-OH-isocaproic, 2-oxo-isovaleric, 2-oxo-3-CH3Val, 2-oxo-iso-caproic, AcLeu, AcIle	MSUD	AACp
3-Hydroxy-isovaleric	Slightly elevated	Valproate treatment	
	► 3-Hydroxy-propionic Isovalerylglycine 3-Methyl-crotonylglycine 3-Methyglutaconic 3-Methyl-3-OH-glutaric		
3-Hydroxy-propionic	Alone	Bacterial infections	AACa
	PG, TG, MC, (2M3KB, 2M3HB, 3HIV)	Propionic acidaemia	
	PG, TG, MC, 3MCG, (2M3KB, 2M3HB, 3HIV)	Biotinidase def or holocarboxylase synthetase def	
	MMA, PG, TG, MC, (2M3KB, 2M3HB, 3HIV)	Methylmalonic aciduria (≠ causes)	AACp+u
Isovalerylglycine	3-OH-isovaleric	Isovaleric acidaemia	Acylcarnitines
	Other acylglycines, glutaric, EMA	GA II, ETHE1	
Lactic	Alone	Bacterial infections	AACp
	2HIB, 2HB, Pyr, KC derivatives	Respiratory chain	Lactate (Redox)
	KC der. + 3MG	Pearson, Respiratory chain	AACp
	OA specific other organic	Organic acidurias	AACp+u
Malonic	Alone	Malonyl-CoA decarboxylase deficiency	
	+Methylmalonic	?	
3-Methyl-crotonylglycine	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, lactate, KC derivatives	Pearson, Respiratory chain	
	3-CH3-glutaric, 3-OH-3-CH3-glutaric, 3HIV	HMG-CoA lyase deficiency	

Table 4.2. *Continued*

Principal OA	Other OAs	Causes of variation	Other investigations
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	15-250 µmol/mmol crea, isolated	SUCLA2/SUCLG1	
	High (>250) with same OAs as in propionic acidaemia	MMA racemase Methylmalonic acidurias: Mutase deficiency CblA, Cblb	
		IF, TCII, CblC, D, D, F, B12 deficiency	AACp (Met↓, Hcy+)
Mevalonolactone	Mevalonic	Mevalonate kinase deficiency	
N-Acetylaspartate	Alone	Canavan disease or Aspartoacylase def	
Orotic		UCDs UMP synthase deficiency (hereditary orotic aciduria)	AACp
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	
Pyroglutamic (oxoproline)	Alone, very high	Glutathione synthetase or oxoprolinase deficiency	
	± High	Secondary: amino acid perfusion, UCD, paracetamol intoxication	
Suberylglycine	► Hexanoylglycine		
Succinylacetone	Several peaks, succinylacetoacetic, 4-OH-phenyllactic, 4-OH-phenylpyruvic, N-AcTyr	Fumarylacetoacetate lyase deficiency (tyrosinaemia type I)	AACp: not specific
Uracil	PyroGlu, Orotate	UCD	AACp
	Thymine	Dihydropyrimidine dehydrogenase deficiency	
Vanillactic	Vanilpyruvic	Transitory in newborns Dopa treatment Aromatic amino acids decarboxylase deficiency	Neurotransmitters in CSF

OA, organic acid; Other OAs: *AcAc*, acetoacetic; *AcIle*, acetylisoleucine; *AcLeu*, acetylleucine; *Ad*, adipic; *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); *3HIV*, 3-hydroxy-isovaleric; *4HB*, 4-hydroxy-butyric; *HG*, hexanoylglycine; *HMG*, 3-hydroxy-3-methyl-glutaric; *IBG*, isobutyrylglycine; *IVG*, isovalerylglycine; *KC*, Krebs cycle; *2MBG*, 2-methylbutyrylglycine; *MC*, methylcitrate; *3MCG*, 3-methyl-crotonylglycine; *3MG*, 3-methylglutaconic; *2M3HB*, 2-methyl-3-hydroxybutyric; *2M3KB*, 2-methyl-3-ketobutyric; *MMA*, methylmalonic acid; *N-AcTyr*, *N*-acetyltyrosine; *nBG* = *n*-butyrylglycine; *2-OH-3CH3Val*, 2-hydroxy-3-methylvaleric; *2OHG*, 2-hydroxyglutaric; *2-OH-isocaproic*, 2-hydroxy-isocaproic; *3-OH-n-But* = 3-hydroxy-*n*-butyric; *3-OHProp*, 3-hydroxypropionic; *PG*, propionylglycine; *Pyr*, pPyruvate; *PyroGlu*, pyroglutamic (or oxoproline); *Seb*. sebacic; *SG*, suberylglycine; *TG*, tiglylglycine; Causes of variation: *UCD*, urea cycle deficiency; *MCT*, medium chain triglycerides; Other investigations: *AAC*, aminoacid chromatography (*p*, plasma; *u*, urine); *SUCLA* succinyl CoA synthetase; *SUCLG* succinyl-CoA ligase

Table 4.3. Assessment of intermediary metabolism over the course of the day

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 deproteinisation (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 (insulin, 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

Table 4.4. Main metabolic abnormalities in lactic acidosis due to inborn errors of metabolism (from [3])

	Gluconeogenesis (G6Pase, FBPase deficiencies)	Glycogenosis type III, VI	PDH deficiency	PC deficiency Hyperlactataemia	KGDH deficiency	Fumarase deficiency	E3 deficiency	Respiratory chain defects
Hyperlactataemia	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
L/P ratio	<15	<15	<10	>30	15-30	<15	15-30	>20
Ketone bodies	↑ At fast or N	Only at fast	Absent	+	+	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 hyperlactataemia. *BCAA*, branched-chain amino acids; *FBPase*, fructose-1,6-bisphosphatase; *G6Pase*, glucose-6-phosphatase; *KB*, ketone bodies; α*KG*, α-ketoglutarate; *KGDH*, α-ketoglutarate dehydrogenase; *3OHB/AcAc*, 3-hydroxybutyrate/acetoacetate; *PC,* pyruvate carboxylase; *PDH,* pyruvate dehydrogenase; *N,* normal; ↑ increased; ↓ decreased; E3 Dihydrolipoamide dehydrogenase

■ **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 with 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.

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 and VI, glucose and lactate change in parallel, with a moderate increase of glucose and lactate in the postprandial state. Fasting hypoglycaemia with postprandial hyperlactataemia is usual in GSD type 0 (glycogen synthase deficiency). Repeated assays are required for glucose and insulin in primary hyperinsulinism, as hyperinsulinaemia 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. Plasma lactate may be persistently elevated, but usually decreases during fasting in patients with pyruvate dehydrogenase (PDH) deficiency and increases in those with GSD type I. 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 resulting from difficult sampling. However, the presence of a lactaturia with an elevation of alanine in blood is very suggestive of a true hyperlactataemia. Lactate measurement in cerebrospinal fluid (CSF) may also be of help in patients with neurological disorders.
3. Measurements of ketone bodies (KB) 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 KB is necessary for the diagnostic and therapeutic evaluation of hypoketotic states, i.e disorders of FAO or ketogenesis (► Chapters 13, 14); data must be interpreted with reference to age and length of fasting (► Fasting Test [below], and also Fig. 1.2).
4. The lactate/pyruvate ratio (L/P) and the 3-hydroxybutyrate/acetoacetate (3OHB/AcAc) ratio reflect the redox states of the cytoplasm and the mitochondrion, respectively, and may provide additional information [3] as follows:
   - L/P increased, 3OH-B/AcAc normal or decreased
   - L/P increased, 3OHB al or decreased: pyruvate carboxylase (PC) deficiency or 3-ketoglutarate dehydrogenase deficiency.
   - L/P and 3OHB/AcAc both increased with persistent hyperlactataemia: RC disorders.
   - L/P normal or low and 3OHB/AcAc normal, with varying hyperlactataemia: PDH deficiency, pyruvate carrier defect.

The usual metabolic abnormalities observed in lactic acidosis due to inborn errors of metabolism are summarised in ◘ Table 4.4 (◘ also Table 1.10).

4.3 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 and acylcarnitines. Other biochemical investigations are needed for the diagnosis of metabolic diseases involving organelles such as lysosomes or peroxysomes. However, these techniques can fail to reveal abnormal metabolic profiles in many patients in whom an inborn error of metabolism (IEM) is suspected, emphasising the need for additional investigatory tools. 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 IEM [4-9], but also of new metabolic diseases. In the last decade, in vitro ^{1}H-NMRS has contributed to the identification of several IEM, 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].

A ^{1}H-NMRS spectrum provides a characteristic 'fingerprint' of almost all proton-containing metabolites. Using three spectral parameters, i.e. chemical shift, spin-spin coupling and signal intensity, ^{1}H-NMRS can be used for the identification and quantification of proton-containing metabolites in urine, serum, plasma or CSF. Briefly, (1) the chemical shift corresponds to the resonance position and helps discriminate the ^{1}H-NMR spectra of molecules even when they share very similar chemical structure; (2) the spin-spin coupling refers to the splitting of the resonances into two or more components and results from the interaction between one or more equivalent protons with their neighbouring protons; and (3) the signal inten-

sity of a resonance, or the peak area, is proportional to the number of protons contributing to the signal and therefore to the concentration of the corresponding molecule. 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, dimethyglycine and trimethylamine *N*-oxide in urine and (2) *N*-acetylneuraminic acid, *N*-acetylaspartylglutamic acid, citric acid, creatine, creatinine and myoinositol in CSF [15].

Overall, the detection limit is in the low micromolar range in the less crowded regions of the spectrum. The detection limit depends on the field strength of the NMR spectrometer, the splitting of the resonances, the number of protons contributing to a resonance, and the region of the spectrum in which the resonance is observed and which is more or less crowded by other resonances. 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. All samples should be kept frozen, preferably at -80°C, until NMRS analyses. A minimal sample volume of 500 µl is required, but 1 ml is preferable. The sample preparation consists in (1) deproteinisation of serum, plasma or CSF samples using a 10-kD filter to eliminate the broad resonances of proteins that interfere with the detection of smaller metabolites; (2) adding a known concentration of an internal reference called TSP (trimethylsilyl-2,2,3,3-tetradeuteropropionic acid) to calibrate the spectra and to quantify metabolites in serum, plasma or CSF samples; and (3) adjusting the pH, since the chemical shift of most proton containing metabolites is pH dependent; most teams work at acidic, 2.5, or neutral pH.

In conclusion, ^{1}H-NMRS is a simple and rapid biochemical tool that can detect a wide variety of metabolites simultaneously, including some that are not usually detected by routine metabolic screening. 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 lipids 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].

4.4 Functional Tests

4.4.1 Fasting Test

- **Indications**

This test [20] has been used for the clarification of hypoglycaemia observed in disorders of gluconeogenesis, FAO and ketogenesis, ketolysis and 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 and enzymatic studies) have ruled out any FAO disorder or as a means of assessing fasting tolerance during the treatment of certain disorders.

- **Procedures**

The fasting test should only be performed in a specialised 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 work-up should be changed accordingly. During the 3 days before the test the patient should be adequately fed and the energy intake appropriate for his/her 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 aged 6-12 months, and 24 h from age 1 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 4.5 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. 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

Table 4.5. 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 after less than 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 Organic acids	0-8 +			16-24 +		

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

Table 4.6. Metabolic profiles during fasting tests in children of different ages (from [4]). 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

for the determination of lactate, KB, 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 4.6).

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 remain very low during the fast.
- Fatty-acid oxidation and ketogenesis defects: glucose <2.8 mmol/l, increased free fatty acids (FFA) and low

KB, with FFA/KB ratio >2 (normal <1) and glucose × total KB <4.
- Gluconeogenesis defect: glucose <2.8 mmol/l and lactate >3.0 mmol/l simultaneously.
- Ketolysis defect: KB already high in the basal state and increasing 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 decreasing during the test.
- 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 with ACTH <80 pg/l.
- Human growth hormone (hGH) deficiency: glucose <2.8 mmol/l and hGH <10 ng/ml simultaneously.

Urine measurements. The best approach is to compare the results of the last period with those of the first.

Complications

Hypoglycaemia, metabolic acidosis, cardiac dysrhythmia, cardiomyopathy and organ failure can all arise as complications of the test. Fluids and medication must be immediately available in the patient's room.

4.4.2 Glucose Loading Test

Indications

This test is used for elucidation of hypoglycaemia or hyperlactataemia of unknown origin.

Procedures

The test should follow a 3- to 8-h period of fasting, depending on 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 then every 30 min thereafter for 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, KB 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) [21]. 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 respiratory chain (RC) disorders [2, 22]. Any increase in lactate must be compared against control values. The L/P ratio, normally around 10:1, is usually increased in PC deficiency and in mitochondrial disorders and is normal or low in PDH deficiency and in mitochondrial pyruvate carrier defect.
- Ketone bodies: KB may increase paradoxically in PC deficiency (with a low 3OHB/AcAc ratio) and in RC disorders (with a high 3OHB/AcAc ratio). Fasting KB are very low in hyperinsulinism.
- The glucagon test, which was formerly widely used for the investigation of hypoglycaemia, has now been largely abandoned as it can be dangerous in conditions associated with fasting hypoglycaemia and in those with catabolic decompensation.

Complications

In patients with PDH deficiency, a glucose load may precipitate lactic acidosis. The test should be stopped if plasma glucose drops below 2.6 mmol/l. The complete metabolic profile should be taken at that time.

4.4.3 Protein and Allopurinol Loading Tests

Indications

These tests are used for the detection of late-onset forms of ornithine transcarbamoylase 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 and urinary orotic acid) are normal. They may also be used to detect heterozygotes in the family of an affected patient [23, 24]. However, for this last indication they have now been superseded by molecular analysis.

■ **Procedures**

Three procedures have been used:

1. Acute protein loading (1 g/kg) test.
2. Allopurinol loading test [9]: 100 mg in children <6 years, 200 mg in children aged 6-12 years, 300 mg in patients >12 years.
3. Combined protein + allopurinol loading test.

The patient should avoid caffeine, tea, chocolate and 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 five fractions: before the test, and then 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 (high-performance liquid chromatography; HPLC).

A fourth procedure may be used in patients in whom 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 involves 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.

■ **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 as a result of 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 [25].

4.4.4 Exercise Test

■ **Indications**

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

- A nonischaemic forearm exercise test [26, 27].
- Bicycle ergometer test [28].
- Treadmill test.

The best exercise test for the widest age span is the treadmill test. The exercise test is also suitable for assessing the results of treatment.

■ **Procedures**

The forearm test and the bicycle test are only applicable in adults and older children who are able to adhere to the protocol and to ride a bicycle. 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-ischaemic modification have been abandoned and replaced by a more accurate nonischaemic test [26, 27].

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 [28].

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 resulting from defects of glycolysis and in those with defects of the citric acid cycle and the respiratory chain. It occurs later in patients with FAO defects (after the exhaustion of energy from glycogen via aerobic and anaerobic glycolysis). Interpretation of the results of each exercise test should take account of this time sequence. 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 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 rarer 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).

Specific acylcarnitine accumulation can be observed in fatty acid oxidation disorders. An elevation of CK and K^+ reflects abnormal myolysis.

4.5 Post-mortem Protocol*

Since the first description of a post-mortem protocol by Kronick [29], some refinements have become available to enhance the diagnostic value of the original recommendations [30, 31]. In the protocol given below, the time schedule for proper preservation of specimens determines the sequence of the diagnostic procedures.

4.5.1 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 -70°C. Part of the liver biopsy should be fixed for histological and electron microscopic investigation prior to freezing (► Section 4.5.5). 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.

4.5.2 Cells and Tissues for Chromosome and DNA Investigations

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

4.5.3 Skin Fibroblasts

At least two biopsies (diameter 3 mm) are taken under sterile conditions as early as possible: one from the forearm and one from the upper leg (fascia lata, ► above). Although a delay decreases the chance of successful fibroblast cultivation, fibroblasts can often be cultivated even from biopsies taken many hours after the death. A biopsy may also be taken from the pericardium in case of delayed autopsy. These samples are conserved in culture medium or, alternatively, on sterile gauze wetted in sterile saline and sealed in a sterile tube for 1 night at room temperature.

* We acknowledge Jan Huber who wrote this part in the 4th edition

Table 4.7. 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
Haematology: 0.5 ml in EDTA tube
Blood gases: 0.5 ml on heparin-coated syringe (eject air bubble, cap syringe immediately)
Electrolytes, urea, creatinine, urate, total protein, liver function tests: 1-2 ml (centrifuge after clotting)
Glucose: 0.3 ml fluoride-heparin cup (dry heparin and fluoride salts, no solution)
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 cup (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)
Urine
pH, amino acids, organic acids, ketone bodies, lactate, reducing substances: 5 ml (at least), freeze at -20°C
Cerebrospinal fluid
Cells, protein, glucose: 0.5 ml in plastic tube
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
Amino acids: 0.5 ml in plastic tube
Culture: 1 ml in sterile tube

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

4.5.4 Body Fluids for Chemical Investigations

Plasma from the centrifuged blood sample, urine (~10 ml) and CSF (~4 ml) are immediately frozen at -20°C (■ Table 4.7). If no urine can be obtained by suprapubic puncture or catheterisation, the bladder may be filled with 20 ml of saline solution and diluted urine may be harvested. Alternatively, vitreous humour can also be collected (by intraocular puncture) and frozen. This liquid is comparable to blood plasma with respect to its solubility for organic acids. Bile, readily available at autopsy, has been found to be useful material for the postmortem assay of acylcarnitines [32].

Many biochemical parameters are impossible to interpret post mortem owing 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 FAO disorders and for organic acidurias.

4.5.5 Autopsy

The autopsy is important, particularly in undiagnosed patients, where it may give important clues to the underlying disorder. It should be as complete as possible and include the cranium, provided that the parents have given permission. 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 and skin biopsies, and to perform 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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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 and children 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 commonest presentation and the most frequent 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, hereditary fructose intolerance and tyrosinaemia type I are amenable to emergency treatment.
3. *Hypoglycaemia:* Blood glucose levels must be corrected immediately. The three groups of disorders usually implicated are hyperinsulinism, glycogen storage disease (GSD) and mitochondrial fatty acid oxidation (FAO) defects.
4. *Cardiac failure:* In neonates the only treatable disorders are FAO defects.
5. *Primary hyperlactataemia:* This is associated with a general lack of cellular energy and may be due to different enzymatic defects. Some patients may benefit from high-dose vitamin treatment.
6. *Intractable convulsions:* Vitamin responsiveness (pyridoxine, pyridoxal phosphate, folinic acid, biotin) must be assessed systematically.

In older children all these clinical situations can also arise. In particular, any type of coma or acute psychiatric symptoms can be the presenting sign of a metabolic disorder. In addition, children may present with recurrent attacks of unexplained dehydration, abdominal pain, muscle pain and myolysis or peripheral neuropathy.

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 for which specific emergency therapy exists should be available within 24 h.

5.1 General Principles

5.1.1 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 present must be treated.

5.1.2 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 [1-3]. 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 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).

5.1.3 Specific Therapies

Specific therapies can be used for certain disorders. These mainly comprise substrates that enable the excretion of ammonia by alternate pathways (► Chapter 20), carnitine and vitamin supplementation and administration of additional specific drugs [5] (► Appendix A: List of Drugs). All intensive care units should ensure that these are readily available.

5.1.4 Extracorporeal Procedures for Toxin Removal

For those disorders associated with acute metabolic toxicity, such as BCOAs and UCDs, 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 far more efficient than exchange transfusion (ET) or peritoneal dialysis (PD) [6].

5.2 Emergency Management of Particular Clinical Presentations

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

5.2.1 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 [7-9]. The management of children (late-onset coma) is essentially similar to that of neonates [8, 9].

■ Supportive Care

■■ Neurological Deterioration with Ketoacidosis

This is the usual presentation in patients with BCOAs including 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), mild to moderate dehydration (<10% of birth weight) and normal or slightly 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 mEq/l), are seriously dehydrated (by >10% of birth weight), and may have hyperammonaemia (>400 μmol/l), mild hyperlactacidaemia (<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 have a 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 a very 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].

■■ Mildly Affected Patients

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 34 mmol/l of Na^+ (2 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 Patients

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 ce-

rebral 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 70-85 mmol/l of Na^+ (4-5 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 [13-15]. 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.

■ 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. NaCl and KCl should be progressively decreased to 2 g/l and 1.5 g/l, respectively. 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 [16, 17]. 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, 18]. 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. 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 [18]. 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. 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, 16, 19]. 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 + nonurinary losses), which are 50-60% below the normal requirements (protein accretion + nonurinary losses + urinary losses) and consequently less than the RDA [20]. 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 (0.2-0.3 IU/kg/h) used in association with large amounts of glucose provided by TPN may be useful [4, 21, 22]. 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 acidopathies, 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. 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 [23]. Owing to its own toxicity, arginine supplementation should be reduced to its nutritional requirement as soon as acute hyperammonaemia has abated [24]. 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 acidopathies, as there is the theoretical risk of additional intramitochondrial coenzyme A depletion [25, 26]. However, in many metabolic centres sodium benzoate is regularly used, without apparent adverse effects [5, 12, 27]. 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. Enteral sodium phenylbutyrate is employed to provide a source of phenylacetate. However, the use of these drugs must be limited before a precise diagnosis indicating a hyperammonaemia patient is obtained, since glutamine is elevated only in urea cycle defects and is in the low-normal range in organic acidurias [28]. 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 [29].

L-Carnitine is given to compensate for secondary carnitine deficiency caused by urinary excretion of carnitine-bound organic acids [30, 31]. 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 risk of cardiac arrhythmia.

■■ 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 [32, 33].

■■ Additional Drugs

In methylmalonic aciduria, forced diuresis and alkalinisation of urine with sodium bicarbonate help to eliminate methylmalonic acid. In propionic and methylmalonic aciduria, metronidazole suppresses intestinal bacterial propionate production. In isovaleric aciduria and methylcrotonyl CoA carboxylase deficiency, 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 [30, 34].

■■ Extracorporeal Toxin-removal Procedures

In some cases, the situation deteriorates so rapidly that an extracorporeal toxin-removal procedure becomes necessary. Such treatment should be considered if the ammonia concentration exceeds 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, 35-37], continuous veno-venous haemofiltration (CVVHF) [38-40] and hemodiafiltration (CVVHDF) [6, 36] have been shown to be more effective than ET and PD. Extracorporeal membrane oxygenation has been used in driving HD and haemofiltration (HF) [41]. 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:

■■ Exchange Transfusion

Exchange transfusion has only a transient effect and it should only be used in combination with other methods or when it can be undertaken repeatedly or continuously [42-44].

■■ Peritoneal Dialysis

Manual PD requires minimal technical expertise, can be rapidly initiated in any paediatric intensive care unit and can be effective in newborns [42, 45, 46]. 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 [47]. 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 [48]. 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, 36, 38-40, 49, 50].

■■ Haemodialysis

HD is a very effective and rapid method of removing small solutes [6, 37, 43, 51]. 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, 35, 43, 48].

■ 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 the organic acidurias, neutropenia and thrombocytopenia may be present or may develop after the initiation of therapy and may require specific treatment. Repeated assessments for septicaemia must be undertaken and treatment initiated as soon as there is any suspicion of infection.

5.2.2 Liver Failure

Liver failure is a predominant finding in 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, 9). When tyrosinaemia type I is confirmed, to prevent production of toxic metabolites treatment with NTBC, along with a low-phenylalanine and low-tyrosine diet must be started as soon as possible, since some cases have shown an immediate recovery from acute liver failure [52, 53] (► Chapter 18).

5.2.3 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 pathological metabolites may become normal quickly with therapy, adequate samples for metabolic studies (acylcarnitines, glucose, insulin, free fatty acids 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).

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

In these disorders, fasting hypoglycaemia is associated with hyperlactataemia and metabolic acidosis. In GSD

type III, moderate hyperlactataemia is observed after glucose administration. As soon as the blood 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 to suit the diagnosis (► Chapters 6, 9).

- **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 10.

- **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 compromised 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. In long-chain FAO defects there is a risk that toxic acylcarnitines will form, although severe secondary carnitine deficiency may require cautious oral treatment. In other disorders of FAO there may be benefit from early supplementation of carnitine to compensate for primary or secondary carnitine deficiency and to promote the excretion of fatty acylcarnitine esters (► Chapter 13). However, the outcome for patients with medium-chain acyl-CoA dehydrogenase (MCAD) deficiency appears to be excellent without such treatment.

5.2.4 Cardiac Failure

The only treatable disorders that lead to presentation with cardiac failure in the neonatal period are the 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 13).

5.2.5 Primary Hyperlactataemia

Whatever the enzyme defect, most newborns with primary hyperlactataemia present with acute ketoacidosis and dehydration requiring supportive care similar to that described for the BCOAs. 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.

Few strategies are of proven efficacy in congenital lactic acidaemia. A trial should be performed with thiamine (co-factor for the pyruvate dehydrogenase [PDH] complex), riboflavin (co-factor for complex I) and biotin (co-factor 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 means of lowering lactate accumulation in both PDH and respiratory-chain disorders [54]. However, it has little effect on the clinical status. In one patient with the French phenotype of pyruvate carboxylase deficiency, the early administration of triheptanoin allowed survival for several months [55] (► Chapters 12, 15, 17). However, the child collapsed during his next decompensation.

5.2.6 Intractable Convulsions

When seizures are the preponderant or presenting sign, pyridoxine, pyridoxal phosphate [56], biotin and folinic acid [57] 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 hydroxycobalamin, folinic acid, pyridoxine, betaine and/or methionine, depending on the underlying enzymatic defect. GLUT1 deficiency can be 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 chloralhydrate.

5.3 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 [58]. 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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II Disorders of Carbohydrate Metabolism

The Glycogen Storage Diseases and Related Disorders

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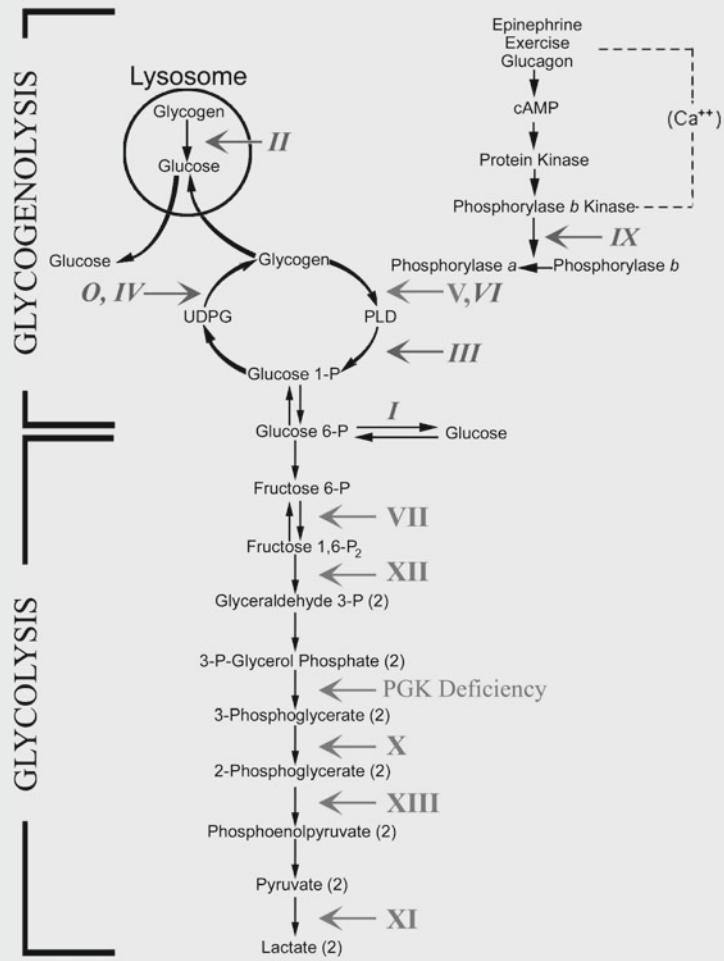
Glycogen Metabolism

Glycogen is a macromolecule composed of up to 60,000 glucose molecules joined into straight chains by α-1-4 linkages and with branching points formed by α-1,6-linkages at intervals of 4-10 glucose residues. Glycogen, the primary energy source between meals, is found in many tissues but is especially abundant in liver and muscle. In the liver, glycogen serves as a glucose reserve for the maintenance of normoglycaemia in times of fasting [1]. In muscle, glycogen provides energy for muscle contraction. Numerous enzymes intervene in the synthesis and degradation of glycogen (◘ Fig. 6.1), and deficiencies of virtually all of these cause glycogen storage disease (GSD) owing to aberrant storage or utilisation of glycogen. The different GSDs are each denoted by a roman numeral that reflects the historical sequence of their discovery, by the deficient enzyme, or by the name of the author of the first description [2].

The clinical features of the GSDs depend on the site of abnormal glycogen metabolism: the liver, muscle, heart, or brain.

Hepatic GSDs present with hepatomegaly and/or hypoglycaemia. GSDs presenting principally with **hypoglycaemia** are GSD I (glucose-6-phophatase deficiency), GSD III (debranching enzyme deficiency, GSD 0 (hepatic glycogen synthase deficiency), and GSD XI (Glut-2 deficiency). GSDs presenting mostly with **isolated hepatomegaly** are GSD VI (phosphorylase deficiency), GSD IX (phosphorylase kinase deficiency) and GSD IV (hepatic branching enzyme deficiency) [3].

Muscle/cardiac GSDs fall into three clinical groups. GSDs presenting with **exercise intolerance** often followed by **rhabdomyolysis** are type V (muscle phosphorylase deficiency) and type VII (phosphofructokinase deficiency). GSD X, GSD XII, and GSD XIII also belong in this group, but these are extremely rare.



◘ **Fig. 6.1.** Scheme of glycogen metabolism and glycolysis. *PGK*, Phosphoglycerate kinase; *P*, phosphate; *PLD*, phosphorylase limit dextrin; *UDPG*, uridine diphosphate glucose. *Roman numerals* indicate enzymes whose deficiencies cause liver (*italics*) and/or muscle glycogenoses: **0** glycogen synthase; **I**, glucose-6-phosphatase; **II**, acid maltase (α-glucosidase); **III**, debranching enzyme; **IV**, branching enzyme; **V**, myophosphorylase; **VI**, liver phosphorylase; **VII**, phosphofructokinase; **IX**, phosphorylase-b-kinase; **X**, phosphoglycerate mutase; **XI**, lactate dehydrogenase; **XII**, fructose-1,6-bisphosphatase aldolase A; **XIII**, β-enolase

GSDs presenting with **myopathy/cardiomyopathy** are type IIa (lysosomal acid maltase deficiency) and type IIb (lysosomal–associated membrane protein 2 deficiency). The extremely rare myopathic forms of GSD 0 and glycogenin 1 deficiency also fit in this category. Unlike the other forms of GSD, GSD III and GSD IXb are the only types that affect both the liver and muscle. A rare GSD presenting with **adult cardiomyopathy** and **Wolff-Parkinson-White syndrome** is represented by the AMP-activated protein kinase (AMPK) deficiency [4].

Brain GSDs present with adult neurodegeneration/epilepsy syndromes associated with accumulation of polyglucosan bodies and encompass several disorders. In brain, all enzymes for synthesis (branching and glycogen synthase) and catabolism (debranching and phosphorylase) are present in both astrocytes and neurons. However, there is no glycogen synthesis in neurons. In astrocytes, glycogen is synthesised and degraded for emergency energy needs 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. Consequently lactate can be used as a potent alternative fuel to glucose in the neurons of GSD patients, especially in case of hypoglycaemia**.** Recent work on myoclonus epilepsy with Lafora bodies (Lafora disease) suggests that this is a glycogenosis, probably due to abnormal glycogen synthesis [5].

6.1 Liver Glycogenoses

The liver GSDs comprise GSD I, the hepatic presentations of GSD III, GSD IV, GSD VI, the liver forms of GSD IX, and GSD 0. GSD I, -III, -VI, and -IX present with hypoglycaemia, marked hepatomegaly, and retarded growth [6]. GSD I is the most severe of these four conditions, because both glycogenolysis and gluconeogenesis are impaired. Patients with GSD III have a syndrome that includes hepatopathy, myopathy and often cardiomyopathy. Unlike the other hepatic forms of GSD, GSD IV usually manifests in infancy or childhood as hepatic failure with cirrhosis leading to end-stage liver disease. GSD VI and the hepatic forms of GSD IX are classically the mildest forms, but there is recent evidence that optimised therapy decreases the rate of liver pathology and improves growth. GSD 0 presents in infancy or early childhood with fasting ketotic hypoglycaemia contrasting with postprandial hyperglycaemia and hyperlactataemia. It is the only hepatic form of GSD without hepatomegaly [3]. The muscle forms of GSD III and -IX are also discussed in this section. Fanconi-Bickel syndrome, which is due to deficiency in GLUT2, is a further cause of glycogen storage, hepatomegaly and fasting hypoglycaemia. This disorder is also associated with postprandial hyperglycaemia and renal tubular dysfunction and is discussed in ► Chapter 11.

6.1.1 Glycogen Storage Disease Type I (Glucose-6-Phosphatase or Translocase Deficiency)

GSD I, first described by von Gierke, comprises GSD Ia caused by deficiency of the catalytic subunit of glucose-6-phosphatase (G6Pase) and GSD Ib caused by a deficiency of the endoplasmic reticulum (ER) glucose-6-phosphate (G6P) translocase [7]. There is controversy about the existence of ER phosphate translocase deficiency (GSD Ic) and ER glucose transporter deficiency (GSD Id) as distinct entities. In this chapter, the term GSD Ib includes all GSD I non-a forms.

▪ Clinical Presentation

Individuals with GSD I usually present at 3-6 months of age with seizures, lethargy, failure to thrive, tachypnoea and developmental delay due to profound hypoglycaemia and lactic acidosis associated with increased intervals between meals and intercurrent illness. Affected infants are usually fussy and unable to sleep through the night without feeding. Hyperlipidaemia and hyperuricaemia are present due to shunting of metabolites through alternate pathways. A protuberant abdomen, truncal obesity, rounded doll-like face, hypotrophic muscles and growth delay are conspicuous clinical findings when patients are diagnosed beyond infancy [8].

Type GSD I occurs in approximately 1 in 100,000 individuals. About one in eight GSD I patients has type Ib [9]. Patients with GSD Ib have symptoms similar to those of patients with GSD Ia, but with the addition of neutropenia and inflammatory bowel disease (IBD) [10]. Neutropenia is a consequence of disturbed myeloid maturation and is also associated with functional defects of circulating neutrophils and monocytes, including impaired motility and migration and impaired metabolic burst [11]. The neutropenia can be either cyclic or constant, and approximately two thirds of patients will have the initial episode of neutropenia before 12 months of age. While the severity of the neutrophil dysfunction is variable, recurrent bacte-

rial infections (predominantly from *S. aureus, S. pneumoniae* and *E. coli)* and oral ulcers are common [12]. An IBD resembling Crohn's disease develops in the majority of patients by the teenage years, which classically is worst in the small intestine [13]. IBD is usually the major cause of morbidity in patients with GSD Ib. Neutropenia and impaired neutrophilic function are thought to be involved in the pathogenesis of IBD, but it continues to occur frequently in GSD Ib patients even during granulocyte colony-stimulating factor (GCSF) therapy.

■ Metabolic Derangement

Among the enzymes involved in hepatic glycogen metabolism G6Pase is unique, since its catalytic site is situated inside the lumen of the ER. This means that its substrate, G6P, must cross the ER membrane and requires a transporter. There is still debate over different proposed models of G6Pase, over the existence of additional transporters for its products, phosphate and glucose [14] and over the existence of GSD Ic (putative ER phosphate/pyrophosphate transporter deficiency) and GSD Id (putative ER glucose transporter deficiency). In particular, patients diagnosed by enzyme studies as having GSD Ic have been found to have the same mutations in the G6P translocase gene as in GSD Ib (► Genetics) [15]. The description of a GSD Id patient has been withdrawn [16].

Hypoglycaemia occurs during fasting as soon as exogenous sources of glucose are exhausted, since the final steps in both glycogenolysis and gluconeogenesis are blocked. There is evidence that hypoglycaemia may improve with age owing to the presence of an isoform of G6Pase after puberty, but severe hypoglycaemia can still occur if therapy is delayed [17]. Hyperlactataemia is a consequence of excess G6P that cannot be hydrolysed to glucose and is further metabolised in the glycolytic pathway. This process is intensified under hormonal stimulation as soon as the exogenous provision of glucose fails. Substrates such as galactose, fructose and glycerol need liver G6Pase to be metabolised to glucose. Consequently ingestion of sucrose and lactose results in hyperlactataemia with no to minimal change in the blood glucose concentration [18].

The serum of untreated patients has a milky appearance due to hyperlipidaemia, primarily from increased triglycerides. The hyperlipidaemia responds to intensive dietary treatment [19]. The increased concentrations of triglycerides and cholesterol are reflected in increased numbers of VLDL and LDL particles, whereas the HDL particles are decreased [20]. VLDL particles are also increased in size due to the accumulation of triglycerides. Hyperlipidaemia is a result of both increased synthesis from excess of acetyl-coenzyme A (CoA) via malonyl-CoA, and decreased serum lipid clearance [21]. Decreased plasma clearance is a result of impaired uptake and impaired lipolysis of circulating lipoproteins. Reduced ketone production during fasting is a consequence of the increased malonyl-CoA levels, which inhibit mitochondrial β-oxidation [22].

Hyperuricaemia is a result of both increased production and decreased renal clearance. Increased production is caused by increased degradation of adenine nucleotides to uric acid, associated with decreased intrahepatic phosphate concentration and ATP depletion [23]. Decreased renal clearance is caused by competitive inhibition of uric acid excretion by lactate [24].

■ Genetics

GSD Ia and -Ib are both autosomal recessive disorders. In 1993, the gene encoding G6Pase (*G6PC*) was identified on chromosome 17q21. Today more than 80 different mutations have been reported [25], with a particularly common mutation in the Ashkenazi Jewish population, where 1 in 72 people are carriers [26]. Subsequently, the gene encoding the G6P transporter (*G6PT*) was identified on chromosome 11q23. More than 65 different mutations have been reported [7]. Patients formerly diagnosed by enzyme studies with GSD Ic and the putative Id shared the same mutations in *G6PT* [27]. Recently, however, a GSD Ic patient without mutations in *G6PT* was described, suggesting the existence of a distinct GSD Ic locus [28].

■ Diagnosis

Most patients with GSD I can be diagnosed by means of a combination of biochemical and genetic testing [9]. Patients with GSD I have a classic pattern, with hypoglycaemia and hyperlactatemia noted after a brief fast of 3-4 h and associated hyperlipidaemia and hyperuricaemia. The diagnosis of GSD Ia can be established through sequencing of the *G6PC* gene, while mutation analyses of *G6PT* should be performed first if patients suffer from neutropenia and/or recurrent infections [25]. If no mutation can be identified but the suspicion of GSD I remains, enzyme assays in fresh liver tissue should be considered. GSD Ia is characterised by deficient G6Pase activity in intact and disrupted liver microsomes, whereas deficient G6Pase activity in intact microsomes and (sub)normal G6Pase activity in disrupted microsomes indicates a defect in the G6P transporter [2].

■ Treatment

■■ Dietary Treatment

The goal of treatment is to provide a continuous source of glucose and maintain normoglycaemia (glucose >4 mmol/l [70 mg/dl]) and prevent secondary metabolic

Table 6.1. Biomedical targets in GSDI

1.	Preprandial blood glucose >3.5-4.0 mmol/l (adjusted to target 2)
2.	Urine lactate/creatinine ratio <0.06 mmol/mmol (or urine lactate <0.4-0.6 mmol/l)
3.	Serum uric acid concentration in high normal range for age and laboratory
4.	Venous blood base excess >5 mmol/l and venous blood bicarbonate >20 mmol/l
5.	Serum triglyceride concentration <6.0 (<10.0 mmol/l in adult patients)
6.	Normal faecal alpha-1-antitrypsin for GSD Ib patients
7.	Body mass index <+2.0 SDS (in growing children between 0 and +2.0 SDS)

derangements (Table 6.1). In infancy, normoglycaemia should be maintained by frequent lactose-free formula feeds, which can be enriched with maltodextrin every 1.5-3 h. Continuous overnight feeds through a nasogastric or gastrostomy tube may also be used [29]. Because of the many advantages (emotional, composition, practical) breast milk is, in spite of its high quantity of lactose, not contraindicated. If poor growth, increased hepatomegaly, hypoglycaemia or hyperlactataemia is noted, it may be necessary to change over partly or fully to lactose-free bottle-feeding. After the age of 4 months the feeds can be bound with up to 6% rice flour. Later on, wheat products can be used as well. Supplementary feeding can be started as usual at the age of 6 months, taking into account the limitations of lactose and fructose [30].

In 1982, uncooked cornstarch (UCCS) was introduced as a treatment option, and it allows the duration between feeds to be increased [31]. Uncooked cornstarch can be introduced at 6 months of age, but may not be tolerated until 1 year of age due to deficient pancreatic amylase. Boluses of uncooked cornstarch along with complex carbohydrate-rich frequent meals and snacks are the mainstay of therapy from 12 months to adulthood in North America [32]. Uncooked cornstarch is given mixed in water or artificially sweetened drinks. Doses are given 3- to 5-hourly as tolerated. Recent introduction of extended release cornstarch may prevent the need for frequent cornstarch dosing at night, improving quality of life [33, 34]. Blood glucose levels remain more stable overnight, with extended release cornstarch leading to better metabolic control; use during the day must be individualised, however, as it may not provide glucose rapidly enough for adequate cover of activity. When used appropriately, cornstarch supplementation is not associated with obesity. Overnight continuous feeds can also be used for maintenance of blood glucose concentrations. Both methods can be successfully used, but overnight continuous feeds must be used with caution, since any interruption of the therapy can result in rapid development of severe hypoglycaemia, seizures or even death. In addition, early morning hypoglycaemia may occur following discontinuation of the overnight feed, and breakfast should be ingested within 30 min after the nocturnal feeding has stopped.

Whether continuous feeds or cornstarch therapy is used, the glucose requirement in children can be calculated using the formula:

$$y=0.0014x^3-0.214x^2+10.411x - 9.084$$

where y is milligram glucose per minute and x is body weight in kilograms (ideal body weight should be used in overweight patients).

It is of critical importance that enough therapy is administered to avoid lactic acid formation, but excessive intake of cornstarch or carbohydrate can lead to excessive weight gain, increased hepatomegaly or glucose fluctuations from excessive insulin production. During childhood, frequent assessment of dosing should occur, and home monitoring of glucose and lactate is typically supplemented by in-patient assessment of metabolic control.

Patients with GSD I are treated with a strict dietary regimen. Fructose, sucrose and lactose intake should be restricted since these sugars are taken up by the liver and lead to glycogen accumulation or production of uric acid, triglycerides and lactate [35]. Fat should be restricted to less than 30% of total caloric intake, with cholesterol intake of less than 300 mg. Attention should be paid to the inclusion of polyunsaturated (essential) fatty acids in the diet. Protein intake should meet general recommendations, but excessive protein intake can exacerbate hyperuricaemia. Vitamin and mineral deficiencies might develop due to a restrictive diet. In particular, zinc, iron, calcium and vitamins B, C and D are commonly deficient, and supplementation with a multivitamin is therefore recommended. Additional iron supplementation may be required owing to insufficient intake of iron and abnormal hepatic hepcidin regulation [36]. Most patients will require extra vitamin D and calcium because of to the lack of dairy products in the GSD diet [37], and there is recent evidence that medium-chain fatty acid supplementation may improve growth and markers of metabolic control [38].

All patients with GSD I should have an emergency protocol in place for intercurrent illnesses. During illness, continuous feeds with a high-carbohydrate formula (e.g. Tolerex) can be used in an attempt to avoid hospitalisation. If hypoglycaemia or severe lactic acidosis occurs,

intravenous administration of 10% dextrose solution should be started at 1.25-1.5 times maintenance values to maintain glucose above 4 mmol/l (70 mg/dl). Once the acidosis has resolved, intravenous fluids should be tapered off slowly to avoid hypoglycaemia being induced by the increased insulin state.

■■ Pharmacological Treatment

Diet and nutritional supplements remain the mainstay of treatment for GSD. Pharmacological interventions are sometimes required to prevent or treat complications of GSD. With optimal control, hyperlactataemia abates. When elevated lactate persists despite attempts to improve control, *sodium bicarbonate* can be used to buffer lactate in order to keep plasma bicarbonate >20 mmol/l. Bicarbonate also induces alkalisation of the urine, thereby diminishing the risk of urolithiasis and nephrocalcinosis. While bicarbonate therapy may help prevent kidney stones, it is no longer the preferred treatment for prevention of this complication. Progressive hypocitraturia develops with age [39], so that alkalisation with *citrate* (typically *potassium citrate*) is even more beneficial in preventing or ameliorating renal calcification.

Uric acid is a potent radical scavenger, and it may be a protective factor against the development of atherosclerosis [40]. Consequently, it is recommended that serum uric acid be maintained within the high normal range. When levels get elevated, however, patients may be prone to development of gout and urate nephropathy, and treatment with a *xanthine-oxidase inhibitor* (*allopurinol*) should be considered.

With improved therapy, GSD nephropathy is becoming less common, even though hyperfiltration occurs almost universally in this population [41]. If control is not optimal, however, focal segmental glomerulosclerosis may develop, which manifests as hypertension, microalbuminuria, proteinuria and then decreased creatinine clearance. If persistent microalbuminuria is present, a (long-acting) *angiotensin-converting enzyme* (ACE) inhibitor *or angiotensin II receptor antagonist* should be started to reduce or prevent further deterioration of renal function [42, 43]. Protein intake should not exceed RDA recommendations.

Hypertriglyceridaemia is also related to metabolic control, and it usually can be controlled with optimised dietary management [44]. When significant hyperlipidaemia is present (triglycerides over 1000 mg/dl [11.4 mmol/l]) *triglyceride-lowering therapies* (nicotinic acid, fibrates, fish oil) are recommended to reduce the risk of pancreatitis. Studies are conflicting regarding the risk of atherosclerosis in GSD I [45-47]. *Cholesterol-lowering drugs* are not indicated in younger patients. In adult patients, however, progressive renal insufficiency may worsen the hyperlipidaemia and atherogenecity, and in such cases *statins* may be indicated, although there is at present no evidence of their efficacy in this condition. It is critical to always optimise dietary treatment first.

Growth hormone therapy is strictly contraindicated in this condition. Poor growth in glycogen storage disease is a result of chronic acidosis, and near-normal growth occurs with optimal metabolic control [48]. In addition to being unnecessary because there is no growth hormone deficiency, growth hormone increases glycogenolysis and worsens metabolic control. As a result, growth hormone therapy does not improve final height, and may increase the rate of complications. Similarly, neither *oestrogen* nor *testosterone* is indicated to enhance pubertal development, as they do not improve final height scores and they may stimulate hepatic adenoma formation. A barrier method is therefore advised for contraception, but therapy with high doses of progestagen from the 5th to the 25th day of the cycle or with daily administration of low doses of progestagen can be used an alternative [49].

The benefits of prophylaxis with oral antibiotics have not been studied in neutropenic GSD Ib patients. However, prophylaxis with *cotrimoxazol* may be of benefit in symptomatic patients or in those with a neutrophil count $<500\times10^6$/l [50]. *Granulocyte colony-stimulating factor* (GCSF) has been used extensively in the GSD Ib population from 1989. Limitation of the use of GCSF to one or more of the following indications is advised: (1) a persistent neutrophil count below 200×10^6/1; (2) a single life-threatening infection requiring antibiotics intravenously; (3) serious IBD documented by abnormal colonoscopy and through biopsies; and (4) severe diarrhoea requiring hospitalisation or disrupting normal life [51]. Toxicity related to GCSF therapy has been common in the GSD population, and use of the lowest possible dose to prevent infections is recommended. A starting dose of 2.5 μg/kg daily or every other day is recommended, and the dose should be adjusted based upon the clinical response and not the absolute neutrophil count. Complications appear to be dose related. The most serious frequent complication is splenomegaly including hypersplenism. Reports of acute myelogenous leukaemia [52] and renal carcinoma [53] arising during long-term use of GCSF make stringent follow-up necessary. The risk of malignancy appears very low, however, and routine bone marrow aspiration or biopsies are no longer recommended.

■ Follow-up, Complications, Prognosis, Pregnancy

Intensive dietary treatment with improved metabolic control has led to reduced morbidity and mortality and

to improved quality of life [8, 32, 54]. Long-term cerebral function is normal if hypoglycaemic damage is prevented. Most patients are able to lead fairly normal lives, but patients may develop complications of different organ systems [55].

Proximal and **distal renal tubular** and **glomerular functions** are at risk [42, 43, 56]. Proximal renal tubular dysfunction is observed in patients with poor metabolic control and improves after the start of intensive dietary treatment [57]. However, distal renal tubular dysfunction can occur even in patients with optimal metabolic control and may lead to hypercalciuria and hypocitraturia [39, 58]. Progressive glomerular renal disease starts with a silent period of hyperfiltration that begins in the 1st years of life [56]. Microalbuminuria and hypertension may develop at the end of the 1st or in the 2nd decade of life, and this is an early manifestation of the progression of renal disease [59]. In the setting of suboptimal control, proteinuria can worsen, and deterioration of renal function leading to end-stage renal disease in the 3rd-5th decade of life can occur. The similarities in the natural history of renal disease in GSD I and of nephropathy in insulin-dependent diabetes mellitus is striking. The pathogenesis, however, is still unclear. As in diabetic nephropathy, ACE inhibitors should be started if microalbuminuria persists over a period of 3 months with a moderate dietary restriction of protein and sodium [43]. Haemodialysis, continuous ambulatory peritoneal dialysis and renal transplantation are therapeutic options for end-stage renal disease in GSD I.

Hepatic adenomas remain the most common complication in GSD I, and they occur in 70% of patients irrespective of dietary management. Adenomas have been described in patients as young as 3 years of age, but most present during puberty [60, 61]. The cause of hepatic adenoma development is multifactorial with both genetic and environmental influences, but recent evidence suggests that the risk of adenoma development is lower in the setting of good metabolic control [62, 63]. A reduction in the size and/or number has been observed in some patients following attainment of optimal metabolic control. Haemorrhage and malignant transformation are possible complications, but most adenomas stop growing following puberty. To screen for adenomas and to follow their size and number, ultrasonography should be performed at least annually. An increase in the size of nodules or loss of definition of their margins necessitate further investigations, such as CT scans or MRI. In addition, serum α-fetoprotein can be used to screen for malignant transformation, but it has not been found to be a sensitive marker for malignant transformation [64]. The management of liver adenomas is either conservative or surgical. In severe cases of adenomas, enucleation or partial liver resection are therapeutic options [65, 66]. Where there is a recurrence of adenomas or suspected malignant transformation, liver transplantation is a therapeutic option provided there are no metastases [67]. Liver transplantation also corrects glucose homeostasis, but it does not correct the neutropenia, neutrophil dysfunction and enterocolitis common in GSD Ib. Notably, immunosuppression may worsen renal function, and there is a high prevalence of renal failure in GSD I patients who have undergone liver transplantation [68].

Osteoporosis is common in both GSD Ia and GSD Ib, developing without abnormalities in calcium, phosphate, parathyroid hormone or vitamin D metabolism [69]. The aetiology is probably multifactoral, including systemic acidosis, elevated cortisol concentrations, delayed pubertal development, inadequate dietary calcium and lack of physical activity [70]. Adequate supplementation with calcium and vitamin D intake is critical, and bone density measurement is recommended every 2 years after puberty [37].

Anaemia is common in both GSD Ia and GSD Ib [8, 32]. Whilst mild anaemia is often a result of iron deficiency, a more severe and unremitting anaemia may be seen in the setting of large hepatic adenomas as a result of inappropriate production of hepcidin, a peptide hormone that controls the release of iron from intestinal cells and macrophages [36]. Hepcidin-associated anaemia is also common in GSD Ib, but the pathogenesis is different. Instead of aberrant production in tumours, hepcidin is induced by IL-6 production from GSD Ib-associated enterocolitis, and severe anaemia in GSD Ib should warrant a gastrointestinal evaluation if inflammatory bowel disease has not been diagnosed [71].

Polycystic ovaries (PCOs) have been observed in adolescent and adult female patients [72]. Their pathophysiology is unresolved, and their effects on reproductive function are unclear. PCOs may cause acute abdominal pain as a result of sudden increase in size and/or vascular disturbances. This should be differentiated from pancreatitis and haemorrhage into a liver adenoma.

Despite severe hyperlipidaemia, **cardiovascular morbidity** and **mortality** are infrequent and, when present, may be related to secondary metabolic changes caused by the progressive renal disease. The preservation of normal endothelial function [45, 46] may result from diminished platelet aggregation [73], increased levels of apolipoprotein E [74], decreased susceptibility of LDL to oxidation – possibly related to the altered lipoprotein fatty acid profile in GSD Ia – and increased antioxidative defences in plasma protecting against lipid peroxidation [45]. A rare vascular complication that may cause more morbidity

and mortality in the ageing patient is **pulmonary hypertension** followed by progressive heart failure [75]. It may develop in the 2nd decade or later, and it appears to be a complication of poor metabolic control.

Successful **pregnancies** have been reported in both GSD Ia and GSD Ib [76, 77]. Close supervision and intensive dietary treatment are necessary, particularly in the 3rd trimester when glucose requirements rise rapidly. Home monitoring of lactate may be beneficial [78], and prematurity has been rare when glucose and lactate concentrations have been kept normal. Adenoma growth during pregnancy has not proved to be problematic, but renal disease can worsen if GSD nephropathy is present [76].

6.1.2 Glycogen Storage Disease Type III (Debranching Enzyme Deficiency)

The release of glucose from glycogen requires the activity of both phosphorylase and glycogen debranching enzyme (GDE). GSD III, also known as Cori or Forbes disease, is an autosomal recessive disorder that is due to deficiency of GDE, which causes storage of glycogen with an abnormally compact structure, known as limit dextrin [1]. Differences in tissue expression of the deficient GDE explain the existence of various subtypes of GSD III [9]. Eighty-five per cent of patients with GSD III have a generalised defect in which enzyme activity is deficient in liver, muscle, heart, leukocytes and cultured fibroblasts, and have a syndrome that includes both hepatic and myopathic symptoms, and often cardiomyopathy (GSD IIIa). About 15% of patients only have symptoms of liver disease and are classified as having GSD IIIb. Subgroups based on the selective deficiency of either the glucosidase activity (GSD IIIc) or of the transferase activity (GSD IIId) are very rare [2, 79].

■ Clinical Presentation

■■ Hepatic Presentation

GSD III presents in the 1st year of life with ketotic hypoglycaemia and hepatomegaly. Hepatic transaminases are markedly elevated and are often in excess of 1000 U/l in the untreated state. Hypertriglyceridaemia is present, but uric acid and lactate concentrations are relatively normal. During childhood, hepatomegaly, short stature, hypoglycaemia, and hyperlipidaemia predominate, and this presentation may be indistinguishable from GSD I [3]. Splenomegaly can be present, but the kidneys are not enlarged and renal function is normal. With increasing age, these symptoms improve in most GSD III patients.

■■ Myopathic Presentation

Clinically evident myopathy may be absent or minimal in childhood, but creatine kinase elevations may be noted as soon as toddlers become ambulatory. Hypotonia and delayed attainment of motor milestones can occur, but clinical manifestations are not usually present until the 2nd decade, when muscle cramps and decreased exercise tolerance manifest. Weakness progresses, worsening with age, and proximal muscles are usually more involved than the distal musculature [80].

Adult-onset myopathies may be distal or generalised. Patients with distal myopathy develop atrophy of leg and intrinsic hand muscles, often leading to the diagnosis of motor neuron disease or peripheral neuropathy [81]. The course is slowly progressive, and the myopathy is rarely crippling. The generalised myopathy tends to be more severe, often affecting respiratory muscles. In the EMG, myopathic features are mixed with irritative features (fibrillations, positive sharp waves, myotonic discharges), a pattern that may reinforce the diagnosis of motor neuron disease in patients with distal muscle atrophy. Nerve conduction velocities are often decreased [82]. Although GDE works hand-in-hand with myophosphorylase and one would therefore expect GDE deficiency to cause symptoms similar to those of McArdle disease, cramps and myoglobinuria are exceedingly rare.

■ Metabolic Derangement

Between meals, insulin levels fall and glucagon secretion increases. These hormones stimulate cleavage of glucose molecules from the terminal strands of glycogen as glucose-1-phosphate via the enzyme glycogen phosphorylase. This process continues until four glucose moieties remain before the α-1,6 bond. The human debranching enzyme has two distinct catalytic activities: 1,4-α-D-glucan 4-α-D-glycosyl transferase and amylo-1,6-glucosidase (AGL). The transferase component transfers the terminal three glucose molecules to the parent chain and the glucosidase component cleaves the α-1,6 bond to release free glucose [1, 2].

The inability to mobilise hepatic glycogen results in hypoglycaemia especially in young patients despite intact gluconeogenesis. Beta-oxidation, however, is normal, and patients develop associated ketosis and hyperlipidaemia [83]. In contrast to GSD I, the fasting blood lactate concentration is normal, but a moderate postprandial lactate elevation (3-5 mmol/l) usually occurs following carbohydrate-rich meals. Elevated levels of serum creatine kinase (CK) and aldolase suggest muscle involvement, but normal values do not exclude the future development of myopathy.

Genetics

The gene for GDE (*AGL)* is located on chromosome 1p21. At least 48 different mutations in *AGL* have been associated with GSD III [84, 85]. GSD IIIb is associated with mutations in exon 3, while mutations beyond exon 3 are associated with GSD IIIa [9]. When all known GSD III mutations are taken into consideration, there is no clear correlation between the type of mutation and the severity of the disease. This makes prognostic counselling based on mutations difficult [86].

Diagnosis

Mutation analysis has become the principal method for diagnosing GSD III if this diagnosis is suspected. Mutations in exon 3 of *AGL* have been associated with GSD type IIIb. Measurement of enzyme activity in skin fibroblasts or lymphocytes can be used to screen for GSD III, but these studies may not be definitive and cannot be used for subtyping. While liver and muscle biopsies are no longer required, they are occasionally still performed as part of evaluation of hepatomegaly or myopathy. Demonstration of abnormal glycogen (limit dextrin) and abnormal enzyme activity in a liver containing glycogen-filled hepatocytes is used to make the diagnosis. Fibrosis is more common than in GSD I. Muscle biopsy typically shows a vacuolar myopathy. The vacuoles contain PAS-positive material and corresponds to large pools of glycogen, most of which is free in the cytoplasm [2].

Treatment

Continuous provision of glucose is required to maintain blood glucose above 4.0 mmol/l (70 mg/dl) [87]. In infancy, frequent formula feeds every 3-4 h maintain normoglycaemia, and some patients require continuous nocturnal drip feeds to prevent hypoglycaemia. No special formulas are required, as lactose and fructose can be utilised but should be limited to individual tailored amounts to avoid over-storage of glycogen. Breast feeding is permitted. Beyond infancy, treatment consists of a high-protein diet (3 g/kg/day during childhood and 2 g/kg/day for adults) in GSD IIIa, along with uncooked cornstarch supplementation, nocturnal feeds or nocturnal drip feeding. Protein can be utilised as gluconeogenesis is intact and provision of adequate dietary protein reverses and may even prevent myopathy and cardiomyopathy [88]. A dose of 1 g/kg of uncooked cornstarch is used initially 3-4 times a day, and the dose of cornstarch is titrated to use the minimum required to maintain normoglycaemia and prevent ketosis. Monitoring blood glucose and ketones helps with optimising dietary control and titrating therapy [87]. In older children and adults, early morning glucose may be normal due to counter-regulatory mechanisms, but monitoring blood glucose concentrations between 2 a.m. and 4 a.m. may detect hypoglycaemia. Fructose and galactose is permitted in GSD III, but excessive amounts of simple sugars and cornstarch should be avoided since they may lead to worsening of the hepatomegaly and cardiomyopathy.

Complications, Prognosis, Pregnancy

Hepatic adenomas occur in approximately 10% of adults with GSD III, and cases of hepatocellular carcinoma have been reported [60, 89]. Hepatic fibrosis can occur, and some adult patients develop cirrhosis, portal hypertension and, rarely, liver failure. Liver transplantation has been performed in patients with end-stage cirrhosis and/or hepatocellular carcinoma [90], but it is not curative since in GSD IIIa the heart and muscle remain untreated [68].

In GSD IIIa, myopathy typically becomes more prominent in the 3rd to 4th decades of life, manifesting as slowly progressive muscle weakness. Exercise causes elevations in serum creatine kinase and aldolase concentrations. Patients with muscle involvement can develop cardiac complications. Concentric left ventricular hypertrophy, detectable by echocardiography, develops after puberty; however, ventricular function is usually normal [91]. Severe cardiac dysfunction and arrhythmias can occur, and rare cases of severe cardiomyopathy in infants and children have been reported [92]. Pregnancies have been successful in GSD III, but monitoring of glucose and ketones is recommended due to changing nutritional needs in pregnancy [93].

Patients with GSD III are prone to adverse effects from several medication classes. In particular, use of succinylcholine and other nonpolarising medications during surgery can trigger substantial muscle damage and rhabdomyolysis. Beta-blockers and statins are not recommended, and they must be used with extreme caution if required [87].

6.1.3 Glycogen Storage Disease Type IV (Branching Enzyme Deficiency)

GSD IV, or Andersen disease, is an autosomal recessive disorder due to a deficiency of glycogen branching enzyme (GBE). Deficiency of GBE results in the formation of an amylopectin-like compact glycogen molecule with fewer branching points and longer outer chains [2]. The pathophysiological consequences of this abnormal glycogen for the liver still need to be elucidated. Patients with the classic form of GSD IV develop progressive liver disease early in life. The nonprogressive hepatic variant of GSD IV is less frequent, and these patients usually survive

into adulthood. Besides these liver-related presentations there are rare neuromuscular forms of GSD IV [94].

Clinical Presentation

Hepatic Forms

Patients are normal at birth and present generally in early childhood with hepatomegaly, failure to thrive and liver cirrhosis. The cirrhosis is progressive and causes portal hypertension, ascites, and oesophageal varices. Some patients may also develop hepatocellular carcinoma [95]. Life expectancy is limited due to severe progressive liver failure and – without liver transplantation – death generally occurs when patients are 4-5 years of age [90, 96].

Patients with the nonprogressive form present with hepatomegaly and sometimes elevated transaminases. Although fibrosis can be detected in liver biopsies, this is apparently nonprogressive. No cardiac or skeletal muscle involvement is seen. These patients have normal parameters for growth.

Neuromuscular Forms

Neuromuscular forms can be divided into four clinical presentations according to the age of onset. A neonatal form, which is extremely rare, presents as fetal akinesia deformation sequence (FADS), consisting of arthrogryposis multiplex congenita, hydrops fetalis and perinatal death. A congenital form presents with hypotonia, cardiomyopathy and death in early infancy. A third form manifests in childhood with either myopathy or cardiomyopathy. Lastly, the adult form may present as a myopathy or as a multisystemic disease also called adult polyglucosan body disease (APBD) [97] (► Section 6.3.2).

Muscle biopsy in the neuromuscular forms shows the typical foci of polyglucosan accumulation, which are intensely PAS-positive and diastase-resistant. Similar deposits are seen in the cardiomyocytes of children with cardiomyopathy and in motor neurons of infants with a Werdnig-Hoffmann-like presentation [98].

Metabolic Derangement

Hypoglycaemia is rarely seen, only occurring when liver cirrhosis is advanced and liver failure sets in. The clinical and biochemical findings under these circumstances are identical to those typical of other causes of cirrhosis, with elevated liver transaminases and abnormal values for blood clotting factors, including prothrombin and thromboplastin generation time.

Genetics

The *GBE* gene has been mapped to chromosome 3p14. Three important point mutations, R515C, F257L and R524X, were found in patients with the classic progressive liver cirrhosis form [99]. In patients with the nonprogressive liver cirrhosis form, the Y329S mutation has been reported. This mutation results in a significant preservation of GBE activity, thereby explaining the milder course of the disease. Interestingly, the mutation found in patients with APBD [97] also appears to be relatively mild, which may explain the late onset of this disorder.

Diagnosis

The diagnosis is usually not suspected until histological examination of a liver or muscle biopsy which shows large deposits that stain with periodic acid-Schiff but are partially resistant to diastase digestion. Electron microscopy shows accumulation of fibrillar aggregations that are typical for amylopectin. The enzymatic diagnosis is based on the demonstration of GBE deficiency in liver, muscle, fibroblasts or leukocytes. Prenatal diagnosis is possible using DNA mutation analysis in informative families, but it is difficult to determine by measuring the enzyme activity in cultured amniocytes or chorionic villi because of high residual enzyme activity.

Treatment

There is no specific dietary or pharmacological treatment for GSD IV. Liver transplantation is the only effective therapeutic approach at present for those with the classic progressive liver disease [90, 96] For patients with hypoglycaemia, cornstarch can be prescribed as a late evening feed with the goal of achieving normoglycaemia. Until transplantation is realised dietary treatment similar to that for GSD III will improve the general condition of the patient.

Complications, Prognosis, Pregnancy

The ultimate prognosis depends on the results of liver transplantation, which was favourable in 13 GSD IV patients [96]. The prognosis also depends on the occurrence of amylopectin storage in extrahepatic tissues. This risk seems to be especially high for cardiac tissue. Of 13 patients with GSD IV who underwent liver transplantation, 2 died from heart failure due to amylopectin storage in the myocardium [96]. A positive result of liver transplantation may be the development of systemic microchimerism, with donor cells present in various tissues. This would lead to a transfer of enzyme activity from normal to deficient cells outside the liver. No pregnancies have been reported in classic GSD IV.

Patients with the nonprogressive liver variant have been reported to survive into their mid-forties. With increasing age, liver size tends to decrease and elevated transaminases return to (near-)normal values.

6.1.4 Glycogen Storage Disease Type VI (Glycogen Phosphorylase Deficiency)

GSD VI or Hers disease is an autosomal recessive disorder due to a deficiency of the hepatic glycogen phosphorylase. Phosphorylase breaks the straight chains of glycogen down to glucose-1-phosphate in a concerted action with debranching enzyme. Glucose-1-phosphate in turn is converted into glucose-6-phosphate and then into free glucose [1].

▪ Clinical Presentation

GSD VI is a rare disorder with a generally benign course. Patients are clinically indistinguishable from those with liver GSD type IX caused by phosphorylase kinase (PHK) deficiency and present with hepatomegaly and growth retardation in early childhood. The hallmark for this disease is ketotic hypoglycaemia in infancy, which is present after an overnight fast, but many patients are diagnosed on the basis of hepatomegaly found incidentally during physical examination. Cardiac and skeletal muscles are not involved. Hepatomegaly decreases with age and usually disappears around puberty [100].

▪ Metabolic Derangement

The tendency to hypoglycaemia is not as severe as seen in GSD I or GSD III, and often the hypoglycaemia is unrecognised in this disorder unless testing is performed in the middle of the night or during illness. Hyperketosis is the predominant abnormality, whilst hyperlipidaemia and hepatic transaminase elevation are usually mild. Lactic acid and uric acid are within normal limits [6].

▪ Genetics

Three isoforms of phosphorylase are known, encoded by three different genes. The gene encoding the liver isoform, *PYGL*, is on chromosome 14q21-q22, and over 40 mutations have been described [101, 102]. A common mutation has been described in the Mennonite population [103].

▪ Diagnosis

Mutation analysis is the preferred method for diagnosing GSD VI. Deficient phosphorylase activity can be documented in liver tissue, but a liver biopsy is not recommended if the diagnosis is suspected.

▪ Treatment

Treatment of liver phosphorylase deficiency is symptomatic and consists in preventing hypoglycaemia and ketosis. This is achieved by prescribing frequent meals along with uncooked cornstarch supplementation: one to three times a day and as a late-evening meal [104]. When hypoglycaemia is not a clinical concern, uncooked cornstarch therapy improves growth, energy, stamina and well-being. Lactose and fructose are permitted, but excessive amounts of simple sugars should be avoided as they may increase hepatomegaly.

▪ Complications, Prognosis, and Pregnancy

The prognosis for those with GSD VI is excellent, but improves with aggressive treatment. Without therapy, delayed puberty and osteoporosis can occur related to chronic ketosis. Hepatic adenomas are rare in GSD VI. Alcohol consumption, however, is problematic in this disorder, as it can precipitate a metabolic crisis and severe hypoglycaemia by impairing gluconeogenesis. It may also predispose patients to scarring and cirrhosis. Hypoglycaemia can occur during pregnancy when metabolic needs increase.

6.1.5 Glycogen Storage Disease Type IX (Phosphorylase Kinase Deficiency)

GSD IX, or phosphorylase kinase (PHK) deficiency, is the most frequent glycogen storage disease. According to the mode of inheritance and clinical presentation six different subtypes are distinguished: (1) X-linked liver glycogenosis (XLG or GSD IXa), by far the most frequent subtype; (2) combined liver and muscle PHK deficiency (GSD IXb); (3) autosomal liver PHK deficiency (GSD IXc); (4) X-linked muscle glycogenosis (GSD IXd); (5) autosomal muscle PHK deficiency (GSD IXe); and (6) heart PHK deficiency (GSD IXf), which, however, is now recognised as being due to mutations in the γ2-subunit of AMP-activated protein kinase rather than to PHK deficiency (► Section 0) [9, 105-107].

▪ Clinical Presentation

▪▪ Hepatic Presentation

The main clinical symptoms are hepatomegaly, growth retardation, elevated liver transaminases, hypercholesterolaemia and hypertriglyceridaemia. While this disorder is classically mild, it is the most heterogeneous of the hepatic GSDs, and some children may phenotypically look like GSD I or GSD III patients, with marked hypoglycaemia and transaminase elevation [108].

▪▪ Myopathic Presentation

The myopathic variants present in a form that is clinically similar to a mild form of McArdle disease, with exercise intolerance, cramps and recurrent myoglobinuria in

young adults. Less frequent presentations include infantile weakness and respiratory insufficiency or late-onset weakness. Muscle morphology shows subsarcolemmal deposits of normal-looking glycogen [109].

▪ Metabolic Derangement

The degradation of glycogen is controlled both in liver and in muscle by a cascade of reactions resulting in the activation of phosphorylase. This cascade involves the enzymes adenylate cyclase and PHK. PHK is a decahexameric protein composed of four subunits, α, β, γ, and δ: the α- and β-subunits are regulatory, the γ-subunit is catalytic, and the δ-subunit is a calmodulin and confers calcium sensitivity to the enzyme. The hormonal activating signals for glycogenolysis are glucagon for the liver and adrenaline for muscle. Glucagon and adrenaline activate the membrane-bound adenylate cyclase, which transforms ATP into cyclic AMP (cAMP) and interacts with the regulatory subunit of the cAMP-dependent protein kinase, resulting in phosphorylation of PHK. Ultimately, this activated PHK transforms glycogen phosphorylase into its active conformation, a process that is defective in GSD type IX [1].

▪ Genetics

Two different isoforms of the α-subunit (α_L for liver and α_M for muscle) are encoded by two different genes on the X chromosome (*PHKA2* and *PHKA1* respectively), whilst the β-subunit (encoded by *PHKB*), two different isoforms of the γ-subunit (γ_T for testis/liver and γ_M for muscle, encoded by *PKHG2* and *PKHG1*, respectively), and three isoforms of calmodulin (*CALM1*, *CALM2*, *CALM3*) are encoded by autosomal genes. The *PHKA2* gene has been mapped to chromosome Xp22.2-p22.1, the *PHKB* gene to chromosome 16q12-q13, and the *PKHG2* gene to chromosome 16p12-p11 [110-112].

The most common hepatic variant, XLG or GSD IXa (resulting from *PHKA2* mutations), comprises two different entities: XLG1, the classic type, and XLG2, the less common variant. In XLG1, the PHK activity is deficient in liver and decreased in blood cells. In XLG2, PHK activity is normal in liver, erythrocytes and leukocytes. Therefore, normal PHK activity in erythrocytes or even liver tissue does not exclude XLG. This phenomenon may be explained by the fact that XLG2 is due to minor mutations with regulatory effects on PHK activity, which is not decreased in vitro [105, 113]. While a strict genotype-phenotype relationship has not been elucidated, mutations in the γ-subunit and some in the α-subunit have been associated with a more severe phenotype with severe hypoglycaemia, lactic acidosis, hepatic fibrosis, and cirrhosis [114, 115].

The predominance of affected men with the myopathic presentation suggested that the X-linked α_M-isoform may be involved predominantly, a concept bolstered by reports of mutations in the *PHKA1* gene in two patients [116]. However, a thorough molecular study of six myopathic patients, five men and one woman, revealed only one novel mutation in *PHKA1*, whereas no pathogenic mutations were found in any of the six genes (*PHKA1*, *PHKB*, *PHKG1*, *CALM1*, *CALM2*, *CALM3*) encoding muscle subunits of PHK in the other five patients. This surprising result suggested that most myopathic patients with low PHK activity harbour either elusive mutations in PHK genes or mutations in other unidentified genes [109].

▪ Diagnosis

Assays of PHK in various tissues may not allow for a definitive diagnosis. Where possible, this should be based on the identification of mutations within the different *PHK* genes [9].

▪ Treatment and Prognosis

Treatment of liver phosphorylase deficiency is symptomatic and consists in preventing hypoglycaemia and ketosis. This is achieved by eating frequent meals, along with uncooked cornstarch supplementation to achieve the aforementioned goals. While some children do not need cornstarch, others need dosing similar to that applied in GSD I, and treatment should be individualised [117]. As outlined for GSD VI, prevention of ketosis improves growth and normalises puberty. Alcohol similarly should be avoided. The prognosis is generally favourable for the hepatic types, and more uncertain for the myopathic variants.

6.1.6 Glycogen Storage Disease Type 0 (Glycogen Synthase Deficiency)

Type 0 glycogen storage disease (GSD 0) is caused by a deficiency of the hepatic isoform of glycogen synthase, which leads to a marked decrease in liver glycogen content [118]. While this disorder has decreased hepatic glycogen, it is characterised as a glycogen storage disease since glycogen is not available during periods of fasting, resulting in a phenotype similar to that of the classic glycogenoses.

▪ Clinical Presentation

Children with GSD 0 usually present with ketotic hypoglycaemia found during an illness or a period of fasting. Developmental delay can occur, but neurological sequelae

are uncommon [119]. Growth retardation from chronic ketosis is common, and patients may paradoxically present with hyperglycaemia as they are found postprandially with hyperglycaemia [120]; children tested in the morning after an overnight fast and breakfast have been misdiagnosed as having diabetes because of the presence of hyperglycaemia and ketosis. The lack of physical findings and less severe clinical course has almost certainly led to underdiagnosis of this condition [119, 121].

▪ Metabolic Derangement

The inability to store glucose leads to a unique pattern of postprandial hyperglycaemia associated with hyperlactataemia alternating with fasting ketotic hypoglycaemia. All patients have overnight ketosis, and the presence of ketones in a morning blood sample can be used to determine which patients with ketotic hypoglycaemia warrant consideration of this diagnosis.

▪ Genetics

The gene that encodes GS, *GYS2,* is located on chromosome 12p12.2, and 15 different mutations are known [119, 122]. No dominant mutation has been identified.

▪ Diagnosis

Mutation analysis is the preferred method for diagnosing this condition. Diagnosis of GSD 0 can also be based on the demonstration of decreased hepatic glycogen content and deficiency of the GS enzyme in a liver biopsy.

▪ Treatment

The goal of treatment is to prevent hypoglycaemia and minimise the associated hyperlactataemia and ketosis. Treatment involves a diet high in protein to provide a substrate for gluconeogenesis and low-glycaemic-index complex carbohydrates to minimise postprandrial hyperglycaemia and hyperlactacidaemia. Uncooked cornstarch (1-1.5 g/kg) administered at bedtime prevents morning hypoglycaemia and ketosis. Daytime hypoglycaemia tends to be mild, and snacks administered every 3-4 h often prevent it; a small dose of cornstarch may be beneficial for children and adults who are particularly active.

▪ Complications, Prognosis and Pregnancy

The prognosis for those with GSD 0 is excellent, and complications are rare. Treatment, however, improves growth, energy, and stamina. As with the other ketotic forms of GSD, alcohol consumption can result in hypoglycaemia, and it must be consumed with caution. Hypoglycaemia can occur during pregnancy when metabolic needs increase [123].

6.2 Muscle and Cardiac Glycogenoses

At rest, muscle predominantly utilises 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.

6.2.1 GSDs With Exercise Intolerance Without Cardiac Involvement

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

▪▪ Clinical Presentation

GSD V, the most common muscle glycogenosis, was first described in 1951 by McArdle. It is characterised 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 metabolise free glucose that is mobilised 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 (CPTII) deficiency, GSD V is the second most common disorder leading to episodes of recurrent myoglobinuria in adults [124], although lipin1 deficiency is now also recognised as a relatively frequent cause (► Chapter 35). Neurological evaluation is usually normal between crises, but proximal muscle weakness and wasting occur in approximately 35% of the patients over 40 years of age [125]. Two patterns of muscle weakness may be observed: (1) proximal and symmetrical, or (2) scapulohumeral and asymmetrical. Resting serum CK is consistently elevated in McArdle patients. Clinical vari-

ants of GSD V with a fatal infantile myopathy have been described in a few cases [79]. 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. The gene for the muscle isoform (*PYGM*) has been mapped to chromosome 11q13. The number of known pathogenic mutations has rapidly increased to over 100 [126]. By far the most common mutation in Caucasians is the p.R50X mutation, which accounts for 81% of the alleles in British patients [127] and 63% of alleles in US patients [128]. This mutation has never been described in Japan, however, where a single codon deletion 708/709 seems to prevail [129].

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 [130].

■■ 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 4). Consequently the IFET should be abandoned and replaced by the standardised nonischaemic FET, which has a sensitivity of 100% in McArdle's disease [131]. 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 [132] or (2) ^{31}P-magnetic resonance spectroscopy to demonstrate abnormal alkalinisation after exercise [133]. 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. Muscle biopsy can be avoided in caucasian patients by identification of the common mutation (p.R50X) in genomic DNA.

■■ Treatment

There is no pharmacological treatment, but exercise intolerance may be alleviated by aerobic conditioning programmes [134] or by ingestion of oral sucrose (37 g), which may have a prophylactic effect when taken 5 min before planned activity [135]. 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 [136]. A recent study indicates that work capacity and exercise tolerance are improved after a carbohydrate-rich diet, an effect that needs to be explored in larger controlled trials [137]. Patients should also avoid strenuous efforts and leisure activities that put them at risk, such as swimming far from the shore and mountaineering.

■ Disorders of Glycolysis

Seven enzyme deficiencies affecting the glycolytic pathway have been described. 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 (◘ Table 6.2).

■■ Clinical Presentation

Phosphofructokinase (PFK) Deficiency: PFK deficiency or GSD VII, first described by Tarui, is the more frequent glycolytic disorder. GSD VII is indistinguishable from GSD V, except that there is no second wind phenomenon and exercise intolerance worsens, rather than improves, after a high-carbohydrate meal, explained by the fact that glucose lowers the blood concentration of the alternative muscle fuels, free fatty acids and ketone bodies [138]. There are two clinical variants, one manifesting as a fixed weakness in adult life (although most patients recognise having suffered from exercise intolerance in their youth), the other affecting infants or young children, who have both generalised weakness and symptoms of multisystem involvement (seizures, cortical blindness, corneal opacifications or cardiomyopathy) [79].

Phosphoglycerate Kinase (PGK) Deficiency: PGK is an ubiquitous enzyme, and the clinical presentation of PGK

Table 6.2. Main features of glycogen storage diseases and related disorders

Type (synonym/s)	Defective enzyme or transporter	Main tissue involved	Main clinical features
Disorders presenting primarily with hepatomegaly and hypoglycaemia			
Ia (Von Gierke)	Glucose-6-phosphatase	Liver, kidney	Hepatomegaly, short stature, hypoglycaemia, lactataemia, hyperlipidaemia
Ib or non-1a	Glucose-6-phosphate translocase	Liver, kidney, leukocytes	Same as Ia, neutropenia, infections
III (Cori, Forbes)	Debranching enzyme and subtypes	Liver, muscle	Hepatomegaly, (cardio)myopathy, short stature, hypoglycaemia
IV (Andersen)	Branching enzyme	Liver	Hepato(spleno)megaly, liver cirrhosis, rare neuromuscular forms
VI (Hers)	Liver phosphorylase	Liver	Hepatomegaly, short stature, hypoglycaemia
IX	Phosphorylase kinase and subtypes	Liver and/or muscle	Hepatomegaly, short stature (myopathy), hypoglycaemia
0	Liver glycogen synthase	Liver	Hypoglycaemia
Fanconi-Bickel	GLUT2	Liver, kidney	Hepatomegaly, short stature, hypoglycaemia, renal tubular disease
Disorders presenting primarily with exercise intolerance due to skeletal myopathy			
V (McArdle)	Myophosphorylase	Muscle	Myalgia, exercise intolerance, weakness
VII (Tarui)	Phosphofructokinase	Muscle, erythrocytes	Myopathy, haemolytic anaemia, multisystem involvement (seizures, cardiopathy)
–	Phosphoglycerate kinase	Muscle, erythrocytes, central nervous system	Exercise intolerance, haemolytic anaemia convulsions
X	Phosphoglycerate mutase	Muscle	Exercise intolerance, cramps
XI	Lactate dehydrogenase	Muscle	Exercise intolerance, cramps, skin lesions
XII	Aldolase A	Muscle	Exercise intolerance, cramps
XIII	β-Enolase	Muscle	Exercise intolerance, cramps
XIV	Phosphoglucomutase	Muscle	Exercise intolerance, cramps
Disorders with cardiac involvement			
I (Pompe)	Acid α-glucosidase	Muscle Heart	Myopathy Cardiomyopathy
IIb (Danon)	LAMP-2	Muscle, heart	Cardiomyopathy, myopathy
GSD III	Debranching enzyme	Muscle, heart	Myopathy, cardiomyopathy
Muscle glycogen depletion syndromes	Muscle glycogen synthase, glycogenin	Muscle, heart	Myopathy, cardiomyopathy,
Cardiomyopathy and WPW syndrome	AMP-activated protein kinase (AMPK)	Heart	Cardiomyopathy, dysrhythmia
Disorders with neurodegeneration			
Lafora disease	Laforin/malin complex (neurons)	Brain	Myoclonic epilepsy, dementia, visual loss
Adult polyglucosan disease	Branching enzyme (astrocytes)	Brain, motor neuron, peripheral nerve	Gait disturbance, bladder dysfunction, dementia: infantile, juvenile, adult forms

WPW, Wolff-Parkinson-White syndrome

deficiency depends on the isolated or associated involvement of three tissues: erythrocytes (haemolytic anaemia), central nervous system (CNS), (seizures, mental retardation, strokes) and skeletal muscle (exercise intolerance, cramps, myoglobinuria). The most common clinical manifestations are nonspherocytic haemolytic anaemia, CNS involvement with anaemia and exercise intolerance with recurrent myoglobinuria. Anaemia and myopathy had been reported in only one patient [139].

Other glycolysis enzyme deficiencies are very rare. GSD X or phosphoglycerate mutase (PGAM) deficiency has been described in about a dozen patients, and GSD XI, -XII, -XIII and –XIV, each in fewer or as single cases. The clinical picture is stereotypical: exercise intolerance and cramps after vigorous exercise, often followed by myoglobinuria.

■■ Metabolic Derangement and Genetics

GSD VII (Muscle PFK Deficiency): PFK is a tetrameric enzyme under the control of three genetic loci that code for muscle (M), liver (L) and platelet (P) subunits. Mature human muscle expresses only an M homotetramer (M4), whereas erythrocytes contain five isoenzymes combining the M and the L subunits. In patients with typical PFK deficiency, mutations in *PFK-M* cause total lack of activity in muscle but only partial PFK deficiency in red blood cells, where the residual activity approximates 50% and is accounted for by the L4 isozyme [79].

PGK is encoded by an X-linked gene (*PGK1*) and is expressed in all tissues except spermatogenic cells.

GSD X: PGAM is a dimeric enzyme with a muscle-specific (M) and a brain-specific (B) subunit. Normal adult human muscle has a marked predominance of the MM isozyme, whereas in most other tissues PGAM-BB is the only isozyme demonstrable by electrophoresis [79]. Molecular defects in the *PGAMM* gene have been identified in patients with GSD X.

GSD XI (Muscle Lactate Dehydrogenase [LD] Deficiency): LD is a tetrameric enzyme composed of a muscle-specific subunit (M or A) and a cardiac subunit (H or B). Mutations have been identified in the gene *LDHM* coding for the muscle subunit.

GSD XII (Aldolase A Deficiency): Aldolase exists in three isoforms (A, B and C): skeletal muscle and erythrocytes contain predominantly the A isoform, which is encoded by the gene *ALDOA*.

GSD XIII: β-Enolase is a dimeric enzyme and exists in different isoforms resulting from various combinations of three subunits, α, β and γ. The β-subunit is encoded by the gene *ENO3*.

GSD XIV: Four isoforms of phosphoglucomutase (PGM) are implicated in phosphotransferase reactions during glycolysis and gluconeogenesis. Mutations have been detected in the *PGM1* gene coding for the PGM1 isoform, representing about 90% of the total PGM activity [140].

■■ Diagnosis

Some routine laboratory results are useful for diagnosis, including an increased bilirubin concentration and reticulocyte count, reflecting a compensated haemolysis that may occur in PFK and PGK deficiencies. Hyperuricaemia is commonly found in PFK deficiency and is attributed to excessive degradation of purine nucleotides in the exercising muscles. Discrepancies between a high level of CK and a low level of LDH are suggestive of LDH deficiency.

When a disorder of glycolysis is suspected, the first step in the evaluation of patient should be a forearm exercise test for measurement of lactate and ammonia levels. Absent or blunted lactate production with an abnormal rise of ammonia levels is a characteristic but inconsistent feature, which should always be followed by a muscle biopsy. ^{31}P-NMR spectroscopy allows detection of an abnormal increase in the phosphomonoester peak in PFK and PGK deficiencies, a useful criterion for distinguishing these enzyme deficiencies [133].

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.

Conclusive evidence comes from the biochemical analysis of enzyme deficiencies either on muscle biopsy for all enzymes, or in erythrocytes for PFK, PGK and aldolase A [141-144].

■■ Treatment

There is no specific therapy, and in contrast to GSD V, in PFK deficiency sucrose and high-carbohydrate diet should be avoided. Aerobic exercise might be useful, but clinical studies remain difficult for such rare disorders.

6.2.2 GSDs with Cardiac Involvement

■ Glycogen Storage Disease Type II (Pompe Disease)

GSD II, also named Pompe disease, acid α-glucosidase deficiency or acid maltase deficiency, is the only lysosomal storage disease among the different glycogenoses and 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 [145].

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 enlargement of the tongue and liver, and major motor milestones are not achieved. Patients most often die from cardiopulmonary failure or aspiration pneumonia without reaching 1 year of age [146].

The juvenile forms are characterised 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 often become dependent on 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 [147]. 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 [148, 149].

■■ 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 works showing an autophagic build-up due to dysfunction of endocytic and autophagic pathways in the muscle fibres [150]. 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 the gene 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 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 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. New screening methods for acid α-glucosidase deficiency using assays in dried blood spots have recently been developed [151] 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 [152]. Electromyography may also help in establishing the diagnosis in the myopathic forms of the disease, showing pseudomyotonic 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 of transgenic rabbit and further in CHO cells (alglucosidase alpha). Alglucosidase alpha has been commercially approved since 2006, and two large studies in infants showed major beneficial effects on cardiomyopathy and muscle weakness, with increased survival [153]. Doses of 20 mg/kg by infusion every other week are recommended. However, less than half of the children on enzyme replacement therapy (ERT) gain normal motor function status and become ventilator free [154]. Several factors may limit the efficacy of ERT in children, such as a severe condition of the patient with extensive muscle damage at the start of treatment or the appearance of high levels of IgG antibodies to rhGAA. A double-blind placebo-controlled trial in adults showed improvement of the walking distance and stabilisation of vital capacity after 18 months of treatment [155]. Long-term follow-up 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.

▪ Danon Disease (LAMP-2 Deficiency)

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

▪▪ Clinical Presentation

The disease presents after the 1st decade, and the characteristic clinical features in male patients include cardiomyopathy in all cases and mild skeletal myopathy and mental retardation in approximately 70%. Fundal examination may detect either retinopathy or maculopathy, and these visual abnormalities may be important clues to this diagnosis in patients with unexplained hypertrophic cardiomyopathy [156]. 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 [157].

▪▪ Genetics

Danon disease is caused by mutations in the gene encoding LAMP2 on Xq24.

▪▪ Diagnosis

The diagnosis may be confirmed by the absence of LAMP-2 staining on immunohistochemistry and detection of mutations in the *LAMP2* gene [158].

▪▪ Treatment

No specific therapy is available, but cardiac transplantation should be considered [159].

▪ Glycogen Depletion Syndromes (Muscle Glycogen Storage disease Type 0 or Glycogen Synthase Deficiency 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 [160, 161].

▪▪ 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 0, whilst PAS-positive material lacking the normal ultrastructural appearance of glycogen is present in glycogenin-1 deficiency.

▪▪ Genetics

Glycogenin1 Deficiency. Glycogenin is a 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 [160].

Muscle Glycogen Synthase Deficiency (Muscle GSD Type 0). Muscle glycogen synthase is ubiquitously expressed and encoded by *GYS1* gene, 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 fatiguability 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 [161].

Treatment

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

Glycogen Storage Disease Type III (Debranching Enzyme Deficiency)

Eighty-five per cent of patients with GSD III have a generalised defect in which enzyme activity is deficient in liver, muscle, heart, leukocytes and cultured fibroblasts, and they have a syndrome that includes both hepatic and myopathic symptoms, and often cardiomyopathy (GSD IIIa) (► Section 6.1.2). Some adult forms present with a predominant myopathy.

AMP-activated Protein Kinase (AMPK) Deficiency

Clinical Presentation

Symptoms starts 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 glycogenosis has been reported in a patient with exercise intolerance, high CK levels and a forearm exercise test showing a blunted lactate increase [162].

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 [163]. 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 [164]. Interestingly, molecular analysis performed in babies who had died of cardiac congenital glycogenosis, 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 [165].

Diagnosis

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

Treatment

Treatment requires a pacemaker/defibrillator, and a heart transplant.

6.3 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 [166]. 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.

6.3.1 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. Polyglucosan bodies are also seen in muscle, liver, heart, skin and retina, showing that Lafora disease is a generalised glycogenosis. The mechanisms by which accumulation of abnormally branched glycogen triggers neuronal apoptosis are undetermined [166, 167].

- **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.

- **Diagnosis**

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

- **Treatment**

No treatment is available.

6.3.2 Adult Polyglucosan Body Disease (Astrocytes Branching Enzyme Deficiency)

- **Clinical Presentation**

Adult polyglucosan body disease is a rare disorder characterised by slowly progressive gait disturbance, urinary incontinence, upper and lower motor neuron dysfunction, distal sensory loss and cerebellar dysfunction. Cognitive impairment occurs in about 50% of cases in the later stages of the disease. Several clinical variants, mimicking spinocerebellar ataxia, extrapyramidal disorders, or motor neuron disease, have been described [168-170] MRI may show atrophy of the cervical spine and diffuse confluent hyperintense periventricular and subcortical white matter signal abnormalities involving the pons, medulla, basal ganglia and dentate nuclei. Electrodiagnostic studies typically show sensorimotor axonal peripheral neuropathy, sometimes with demyelinating features.

- **Metabolic Derangement**

The pathological hallmark is the presence of large polyglucosan bodies in the peripheral nerves, cerebral white matter, basal ganglia, cerebellum and spinal cord. Axillary skin biopsy shows polyglucosan bodies in the myoepithelial cells of apocrine glands and may be helpful in confirming the diagnosis. The accumulation of this amylopectin-like polyglucosan is usually ascribed to the deficiency of glycogen branching enzyme (GBE).

- **Genetics**

Adult polyglucosan body disease is usually sporadic, although there are few familial cases with probable autosomal recessive inheritance, primarily in the Ashkenazi Jewish population. In patients from Ashkenazi Jewish families, genetic analysis has identified a homozygous missense mutation (Tyr329Ser) of the *GBE1* gene, but other *GBE1* mutations have also been found in other ethnic groups. There are also several reported cases in non-Jewish patients with normal GBE activity.

- **Diagnosis**

The diagnosis is usually made by sural nerve biopsy or on autopsy.

- **Treatment**

No treatment is available.

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Disorders of Galactose Metabolism

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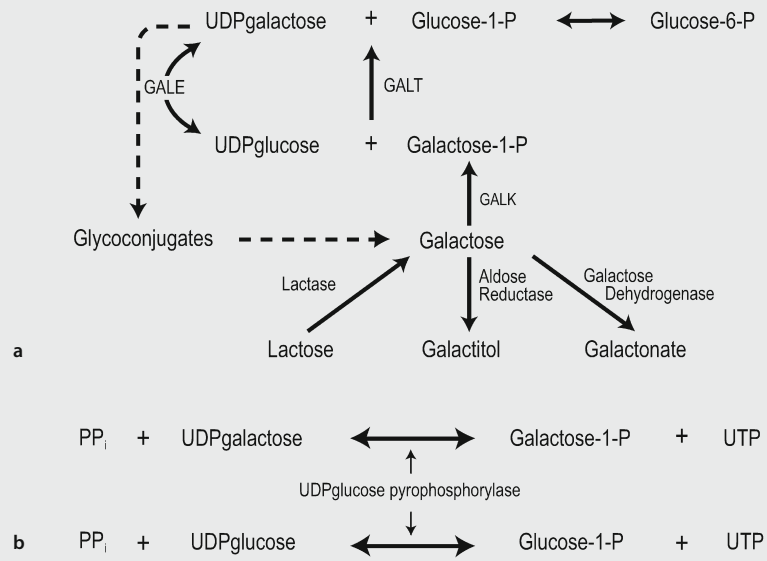
Galactose Metabolism

In nearly all mammals, galactose and UDPglucose are used in mammary tissue to form the disaccharide lactose, which is the principal carbohydrate in milk, providing 40% of its total energy. Although some other foods contain free or bound galactose, milk and dairy products are by far the largest exogenous source of galactose in man. However, endogenous galactose production, independent of dietary intake, is also significant.

Ingested lactose is hydrolysed by lactase in the brush border of the small intestine to form galactose and glucose. Galactose is then absorbed using a sodium/glucose-galactose cotransporter (SGLT1). Although the enzymes involved in galactose metabolism are widespread, including being present in red and white blood cells, the liver is the major organ involved. Galactose is phosphorylated to form galactose-1-phosphate (galactose-1-P) by galactokinase (GALK). Galactose-1-P uridyltransferase (GALT) converts uridine diphosphoglucose (UDPglucose) and galactose-1-P into uridine diphosphogalactose (UDPgalactose) and glucose-1-P. The last is metabolised into glucose-6-P, from which glucose, pyruvate and lactate are formed (not illustrated). Galactose can also be converted into galactitol by aldose reductase, and into galactonate by galactose dehydrogenase. UDPglucose (or UDP-*N*-acetylglucosamine) can be converted into UDPgalactose (or UDP-*N*-acetylgalactosamine) by UDPgalactose 4'-epimerase (GALE). The utilisation of UDPgalactose in the synthesis of glycoconjugates including glycoproteins, glycolipids and glycosaminoglycans, and their subsequent degradation (◘ Fig. 7.1a) may constitute the pathways of endogenous, de novo synthesis of galactose. All four of these uridine sugar nucleotides are used for glycoconjugate synthesis. UDPglucose is also the key element in glycogen production, while UDPgalactose is used for lactose synthesis. The UDPglucose pyrophosphorylase enzyme (◘ Fig. 7.1b) that is primarily responsible for interconversion of UDPglucose and glucose-1-P can catalyse, albeit in a limited way, the interconversion of UDPgalactose and galactose-1-P, and also contribute to endogenous synthesis of galactose.



◘ **Fig. 7.1a, b. a** The important reactions in the galactose metabolic pathway are shown in relation to exogenous, via lactose primarily, and endogenous de novo, synthesis of galactose. The pathways with multiple enzymatic steps are shown by *broken lines*. Carbon skeletons exit the galactose pool via galactokinase (*GALK*)-mediated conversion to galactose-1-phosphate (*galactose-1-P*), aldose reductase-mediated conversion to galactitol, and galactose dehydrogenase-mediated conversion to galactonate. **b** In patients with severe galactose-1-phosphate uridyltransferase (*GALT*) deficiency, there is little or no conversion of galactose-1-phosphate to uridine diphosphate galactose (*UDPgalactose*) and markedly abnormal kinetics for conversion of oral [1-^{13}C]galactose to $^{13}CO_2$ in expired air. However, the UDPglucose pyrophosphorylase enzyme that is primarily responsible for conversion of glucose-1-phosphate (*glucose-1-P*) to uridine diphosphate glucose (*UDPglucose*) can catalyse, albeit in a limited way, the transformation of galactose-1-phosphate to UDPgalactose. The epimerisation of UDPglucose to UDPgalactose by UDPgalactose-4-epimerase (*GALE*), the utilisation of UDPgalactose in the synthesis of glycoconjugates such as glycoproteins and their subsequent degradation (**a**), and/or the UDP glucose pyrophosphorylase (PPi) reaction (**b**), may constitute the pathways of de novo synthesis of galactose [48]

Three inborn errors of galactose metabolism are known. The most important is classic galactosaemia caused by galactose-1-phosphate uridyltransferase (GALT) deficiency. A complete deficiency is life threatening with multiorgan involvement and long-term complications. Partial deficiency is usually, but not always, benign. Uridine diphosphate galactose 4-epimerase (GALE) deficiency exists in at least two forms. The very rare profound deficiency clinically resembles classic galactosaemia. The more frequent partial deficiency is usually benign. Galactokinase (GALK) deficiency is extremely rare and the most insidious, since it results in the formation of nuclear cataracts without provoking symptoms of intolerance. Deficiency of the sodium-dependent monosaccharide carrier SGLT1 causes congenital glucose/galactose malabsorption (► Chapter 11). Fanconi-Bickel syndrome (► Chapter 11) is a congenital disorder of glucose and galactose transport; it is due to GLUT2 deficiency leading to hypergalactosaemia, but its clinical manifestations are renal tubular dysfunction and glycogen storage disease. Other secondary causes of impaired liver handling of galactose in the neonatal period are congenital portosystemic shunting and multiple hepatic arteriovenous malformations.

Whenever you think it possible that an infant may have a disorder of galactose metabolism, stop milk feeding and only then seek a diagnosis!

7.1 Galactose-1-Phosphate Uridyltransferase Deficiency

7.1.1 Clinical Presentation

A diagnosis of classic galactosaemia, which is caused by a complete deficiency of GALT, is most often made following the onset of clinical illness or from a positive newborn screening result. Infants 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 excessive weight loss, poor feeding, vomiting and lethargy. Findings on examination may include jaundice, liver enlargement, excessive bruising or bleeding following blood sampling, and a full fontanelle. Death from septicaemia, particularly with E. coli, may occur. Cataracts are rarely present at birth, but nuclear cataracts usually develop with the onset of clinical illness and may only be detectable on slit-lamp examination. The course and severity of the initial illness may be less severe when milk feeds are temporarily withdrawn and replaced by intravenous nutrition. Occasionally children may first present with a more chronic illness characterised by failure to thrive and developmental delay.

On initial investigations, liver disease is 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). Renal tubular disease is manifest by metabolic acidosis, galactosuria, glycosuria and aminoaciduria. The presence or absence of reducing substances or galactose in the urine should not be relied upon when considering a diagnosis: galactose may be absent from the urine only a few hours after a milk feed, and even if present may be masked by the glycosuria and aminoaciduria associated with renal tubular dysfunction. Hypoglycaemia can occur but is rare. Partial GALT deficiency with around 10% residual enzyme activity in liver may cause clinical illness if untreated; that associated with activities of 25% or more, as occurs in Duarte galactosaemia, is generally asymptomatic.

7.1.2 Metabolic Derangement

Individuals with a profound deficiency of GALT accumulate galactose, galactose-1-phosphate, galactitol and galactonate in body tissues and fluids. Disturbances in the glycosylation of glycoproteins and glycolipids also occur, as demonstrated by transferrin isoelectric focusing, which may be abnormal prior to starting dietary galactose restriction. Cataract formation can be explained by galactitol accumulation. The pathogenesis of the hepatic, renal and cerebral disturbances is less clear, but is probably related to the accumulation of galactose-1-phosphate and (perhaps) of galactitol. Although the amounts of galactose and galactose metabolites decrease significantly with dietary treatment they remain elevated. It is clear that this is largely due to endogenous galactose production rather than dietary sources. Galactose-1-phosphate can be produced from glucose-1-phosphate via the pyrophosphorylase/epimerase pathway (◘ Fig. 7.1), which also provides UDPgalactose available for the biosynthesis of galactolipids and galactoproteins necessary for cell differentiation and growth. Endogenous galactose production occurs in utero and throughout life. It is age related, with higher levels in children than in adults [1, 2]. In adults on a strict lactose exclusion diet, galactose intake was estimated at 20-40 mg/day; at the same time, they produced more galactose endogenously than they consumed in their diets [2, 3].

7.1.3 Genetics

The mode of inheritance is autosomal recessive. The birth incidence of classic galactosaemia is 1 in 40,000-60,000. In Ireland it is 1 in approximately 20,000. The disorder is rare in Asian populations. The GALT gene is on chromosome 9, and over 230 mutations or polymorphisms have been described. (See the following website: http://www.arup.utah.edu/database/galactosemia/GALT_welcome.php [4].) Q188R is the most prevalent mutation in European populations, and it is particularly common in Ireland and Great Britain, where it accounts for over 70% of mutant alleles. S135L is the most frequent mutation in black Africans and African-Americans. Some genotype-phenotype matching is available, with an unfavourable clinical outcome associated with homozygosity for the Q188R mutation in comparison with those homozygous for S135L. Many allelic variants associated with a partial enzyme defect have been reported, but the best known is the D2 Duarte variant, which is due to a *N314D2* GALT gene polymorphism that exists in *cis*, with a small deletion in the 5' flanking region [5]. Variants such as the Q188R/N314D compound heterozygote can be distinguished by enzyme electrophoresis or DNA analysis. The *N314D* Duarte D2 variant when combined with the Q188R mutation (D/G defect) is associated with 25% residual GALT activity and almost always benign. It is relatively common, so that partial transferase deficiency is more frequent than classic galactosaemia.

7.1.4 Diagnostic Tests

Newborn screening (NBS) for galactosaemia is undertaken in many countries by measurement of blood total or free galactose, the transferase enzyme or both, using dried blood spots usually collected 24-48 h after birth. Some programmes quantify galactose-1-phosphate in addition to total galactose. Measurement of galactose alone results in a high rate of false positives and, in infants with a clinical variant form of galactosaemia, may also give false-negative results [6]. In infants with classic galactosaemia, at the time of discovery by NBS the first signs may already have appeared, and the infant been admitted to a hospital, usually for jaundice.

The diagnosis of galactosaemia is confirmed by assaying GALT activity in erythrocytes. An indirect fluorescence blood spot test (the Beutler test) can be used as an initial investigation. False positives occur in infants with G6P dehydrogenase deficiency. More specific quantitative assays are available where GALT activity is measured in erythrocyte lysates. All assays of red cell GALT activity can give a false-negative result if a blood transfusion has been given within the past 2-3 months. In this situation, an assay of urinary galactitol, red cell GAL-1-P mutation analysis, or finding reduced GALT activity in parental blood may provide additional information. Cultured skin fibroblasts can also be used for the enzyme assay. If taken post mortem, liver or kidney cortex may provide diagnostic enzyme information, but these specimens must be adequately collected and frozen.

NBS will also detect infants with partial deficiencies, most commonly D/G galactosaemia. Although D/G galactosaemia appears benign, every newborn with partial GALT deficiency must nevertheless be observed closely in case another allelic variant that may be clinically relevant is present, such as a S135L/S135L genotype in individuals of African descent. Assessment involves quantitation of urine galactitol and of erythrocyte galactose-1-phosphate, investigation of GALT enzyme activity, and GALT gene sequencing, as well as molecular investigation of the parents. Galactose tolerance tests are notoriously noxious to the child with classic galactosaemia and have no place in evaluating the need for treatment of partial deficiencies. Breath tests with [1-^{13}C] galactose may be helpful in the assessment of patients with very rare genotypes [7].

Where newborns are not screened for galactosaemia or when the results of screening are not yet available, diagnosis rests on clinical awareness; however, some infants are detected from the finding of raised blood phenylalanine and tyrosine (as a result of liver disease) assayed as part of the NBS for phenylketonuria.

▪ Prenatal Diagnosis

If the mutation is known within a family, prenatal diagnosis can be performed by analysis of DNA extracted from a chorionic villous biopsy collected at about 11 weeks of gestation. Prenatal testing can also be achieved by measuring GALT enzyme activity directly in chorionic villous cells, in cultured chorionic villous cells or in cultured amniotic fluid cells and by measurement of amniotic fluid galactitol. Restricting maternal lactose intake does not interfere with a diagnosis based on galactitol measurements in amniotic fluid [8].

7.1.5 Treatment and Prognosis

▪ Newborns

Treatment consists in the exclusion of all lactose from the diet. Breast feeding or milk-based formulas must be stopped immediately the disorder is suspected clinically or following a positive newborn screening result, and before confirmatory diagnostic tests are available. Most

newborns are changed to a soya-based formula, although an elemental formula may also be used. Soya contains a small amount of bound galactose and isoflavones, although these have not been shown to have any adverse clinical effects [9]. In the presence of significant liver disease a medium-chain triglyceride containing casein hydrolysate preparation may be tolerated better, but since it contains a small amount of galactose should not be used once the liver disease has resolved. Some infants are seriously ill at diagnosis and will require considerable supportive care, including the management of a coagulopathy and septicaemia. When a lactose-free diet is instituted early enough, symptoms disappear promptly, jaundice resolves within days, cataracts may clear, liver and kidney functions return to normal and liver cirrhosis may be prevented.

▪ Infants and Children

At weaning parents must learn to identify all other sources of lactose and will need assistance from the paediatrician and dietitian, who must have recourse to published recommendations. Parents are advised to prepare meals from basic foodstuffs and to avoid canned food, by-products and preserves, unless they are certified not to contain lactose or dairy products, to read and reread labels and declarations of ingredients, which may change without notification, and to look out for hidden sources of galactose and lactose from milk powder, milk solids, hydrolysed whey (a sweetener labelled as such), drugs in tablet form, toothpaste, baking additives, fillers, sausages, etc. Some Swiss cheeses and certain other hard cheeses are galactose and lactose free, as these sugars are cleared by the fermenting microorganisms [10]. Although soya-based infant formulas normally provide sufficient calcium in infancy, at weaning a fortified soya milk or calcium supplement is necessary. Certain calcium prescriptions that contain lactobionate are a source of galactitol, since beta-galactosidase, which is present in human intestinal mucosa, may hydrolyse the lactobionate.

Even with strict adherence to dietary treatment, a completely galactose-free diet is not possible. Galactose is present in a great number of vegetables, fruits and legumes (beans, peas, lentils etc.), as a component of galactolipids and glycoproteins, in the disaccharide melibiose and in the oligosaccharides raffinose and stachyose [11]. The last two contain galactose in alpha galactosidic linkage, which is not hydrolysable by human small intestinal mucosa in vitro or in vivo, although absorption might occur if the small intestine is colonised by bacteria capable of releasing galactose. In addition, the normal inhabitants of the large colon may facilitate the release of galactose from macromolecules that pass through. However, when compared with endogenous production, it is unlikely that absorption of galactose in the large colon has a significant impact on the expanded whole-body pool of galactose in the patient on a lactose-restricted diet. There is no evidence that restriction of other foods leads to any improvement in outcome.

▪ Older Children and Adults

Current recommendations are that dietary restriction of galactose needs to be continued for life. Single reports and anecdotal information suggest that children and/or adults may suffer cataracts, liver disease and organic brain disease with ingestion of lactose [12] (G.T. Berry, unpublished observations). Milligram amounts of galactose cause an appreciable rise of galactose-1-phosphate in erythrocytes (e.g. ~500 mg of galactose in a 70-kg adult with Q188R/Q188R genotype will acutely increase galactose-1-phosphate by 30% in 8 h); it is possible that the same happens in sensitive tissues, such as brain, liver and kidney. However, it is not possible to define toxic tissue levels of galactose-1-phosphate, and safe amounts of dietary galactose therefore cannot be determined. Patients with relatively increased alternative metabolic pathway activities should have greater tolerance for galactose. Two cases of classic galactosaemia have been reported, and there are also a few anecdotal cases, in which stopping dietary restriction after early childhood has resulted in outcomes that are no worse than those of patients who have continued treatment [13, 14].

▪ Biochemical Monitoring

Erythrocyte Gal-I-P concentration has been the most common biochemical assay used to monitor treatment. The level is often very high at diagnosis and only falls gradually over weeks and months after the initiation of treatment. However, even with good dietary compliance the concentration remains above normal. The usefulness of this assay is open to question, as the level of Gal-I-P has not been shown to correlate with outcome. Other galactose metabolites, including red cell galactitol, urine galactitol and red cell galactonate, are also consistently increased in classic galactosaemia and have been suggested as alternative or additional markers [15]. However, there is currently no data available to demonstrate their superiority over GAL-1-P. Analysis of the glycosylation of plasma transferrin from untreated newborn infants indicates both assembly and processing patterns similar to those seen in congenital disorders of glycosylation (CDG) [16].

▪ Long-term Outcome and Complications

Despite the rapid clinical response to lactose exclusion in newborn infants with classic galactosaemia, long-term

complications are common; these appear to be largely independent of the severity of any initial illness and the strictness of dietary compliance [17-19]. Mild growth retardation, decreased bone density, delayed speech development, verbal dyspraxia, difficulties in spatial orientation and visual perception, and intellectual deficits have been variably described as complications of treated galactosaemia [17, 18, 20-24]. Tremor, ataxia, dystonia and choreic movements are also reported. The quality of life in treated patients has been unfavourably compared with that of those with PKU [25]. Some patients, males in particular, manifest an introverted personality and/or depression.

The complete set of sequelae is not necessarily present in every patient, and the degree of handicap appears to vary widely. Some studies have reported a progressive fall in IQ with increasing age, but this has not been found in others, suggesting that the apparent cognitive decline may be related to the testing instruments [20]. A minority of patients have developed more severe neurological disease with cerebellar dysfunction. The brain MRI may reveal white matter disease and both cerebral and cerebellar atrophy [26]. Fluorodeoxyglucose positron emission tomography (FDG-PET scan) has shown a decrease in cerebral glucose metabolism in certain areas, although the abnormalities have been highly variable [27].

Although the majority of these late complications cannot be prevented, early identification of decreased bone mass by dual-energy X-ray absorptiometry (DXA) and advice on physical activity and ensuring an adequate intake of vitamins D and K1, plus calcium and oestrogen supplementation in girls, may help to reduce the risk of osteoporosis [28].

▪ Fertility and Pregnancy

Ovarian failure occurs in the large majority of women with classic galactosaemia and presents clinically with delayed menarche, primary or secondary amenorrhoea or oligomenorrhoea. In contrast, male gonadal function has not been shown to be adversely affected (for review see [29]). The cause of ovarian damage remains unclear, but it 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) [30]. Since in female patients the number of expected ovulatory cycles is limited, it may be wise to suppress cycles temporarily by birth control medication, which is withdrawn when one of these young women wishes to become pregnant. This is not an established form of therapy, in contrast to chronic oestrogen and progesterone supplementation. Prescription is hampered by the fact that seemingly all drug tablets contain lactose, providing 100 mg or more per treatment day. However, some female patients have received birth control medication containing galactose for many years without any obvious side effects (G.T. Berry, personal observation). Although this is uncommon, a small number of women with classic galactosaemia, including those with Q188R/Q188R genotype, have experienced one or more successful pregnancies and deliveries; some of them subsequently developed secondary amenorrhoea. Galactose metabolite concentrations do not appear to increase significantly during pregnancy, although levels show a transient rise after delivery, peaking within the 1st week and subsequently falling even in those who choose to breast feed [31]. Those infants born to mothers with classic galactosaemia have been normal.

▪ Dietary Treatment in Pregnant Women at Risk

Based on the assumption that toxic metabolites deriving from galactose ingested by the heterozygous mother accumulate in the galactosaemic fetus, mothers may be counselled to refrain from or limit drinking milk for the duration of pregnancy. However, despite dietary restriction by the mother, galactose-1-phosphate and galactitol accumulate in the fetus [32] and in the amniotic fluid [8], presumably as the result of endogenous synthesis. Furthermore, the outcome for infants whose mothers restricted milk intake in pregnancy was no better than for those whose mothers did not [20].

▪ Management of Partial Transferase Deficiency Attributable to D/G Genotype

Infants with Duarte D2 galactosaemia are frequently detected on NBS. Some centres have adopted a pragmatic approach, prescribing a lactose-free formula to all infants discovered by NBS for 1-4 months after birth until erythrocyte galactose-1-phosphate levels normalise and remain normal with a regular lactose-containing diet. This transition may be initiated with a lactose challenge. For example, if at the end of a 1-week trial with a daily supplement of formula containing lactose the erythrocyte galactose-1-phosphate level is below 1 mg/dl the infant will be returned to normal nutrition. Other centres opt for 1 year of treatment and utilise a 1-month challenge with cow's milk. We consider that the large majority of evidence indicates that D/G galactosaemia is a benign condition: in countries without screening for galactosaemia, D/G galactosaemia is not reported associated with any clinical symptoms, and a recent study of 30 children between 1 and 6 years of age did not demonstrate any developmental or clinical pathology [33]. However, some controversy still remains; another study has revealed an excess of affected children who had specific speech and language problems compared with the normal population [34].

■ **Heterozygotes for Classic Galactosaemia**

Heterozygotes for GALT deficiency have not been shown to be at increased risk of premature menopause, presenile cataracts or other disease manifestations [35].

■ **Future Treatments**

Work is in progress to try and develop a specific and safe inhibitor of the galactokinase (GALK) enzyme [36]. If successful, this may allow classic galactosaemia to be transformed into a disease process that would more nearly resemble GALK deficiency. If that were the case, the patients might benefit greatly, but would still be at risk of cataract development. Along with the use of a lactose-restricted diet, patients may benefit from the use of aldose reductase inhibitor (ARI) to block galactose conversion to galactitol [37].

7.2 Uridine Diphosphate-Galactose 4′-Epimerase Deficiency

7.2.1 Clinical Presentation

GALE deficiency varies from an asymptomatic condition only detected as a result of newborn screening for GALT deficiency to a severe disorder that presents with a life-threatening illness in the newborn period. The severe form of GALE deficiency is extremely rare, with a total of five patients from two consanguineous families known [38]. In those infants exposed to lactose the initial clinical presentation has been similar to that in GALT deficiency, with jaundice, weight loss and hypotonia, and vomiting, generalised aminoaciduria and galactosuria. Learning difficulties, sensorineural hearing loss, growth delay, micrognathia, ligamentous laxity and contractures of fingers have been present in the majority of patients. One child died from unexplained liver failure at 4 months of age. Unlike GALT deficiency, GALE deficiency has not been found to prejudice ovarian function. A few patients with an intermediate form have also been described, with findings ranging from a transient illness with seizures, vomiting and hypoglycaemia in response to lactose ingestion through developmental delay to juvenile cataracts [39].

7.2.2 Metabolic Derangement

GALE is essential for the normal biosynthesis of glycoproteins and glycolipids. Complete GALE deficiency is likely to be incompatible with life; even patients with the severe form of the disease have some residual tissue enzyme activity. Asymptomatic individuals have enzyme activity reduced only in red cells (peripheral GALE deficiency), whereas in intermediate and severe forms the reduction is more widespread (generalised GALE deficiency). Red cell GALE activity does not correlate well with that in lymphoblasts and is poor at differentiating between peripheral and generalised forms of the disease. GALE deficiency leads to an accumulation of galactose, galactitol and galactose-1-phosphate, but in contrast to GALT deficiency there are an increase in UDP-GAL and a decrease in UDP-Glc (◘ Fig. 7.1). As in GALT deficiency, abnormal glycosylation of proteins that appears to be dependent, at least in part, on lactose consumption has been reported in severe GALE deficiency [38] and is thought to be a secondary biochemical complication not primarily related to the genetic defect.

7.2.3 Genetics

Epimerase deficiency is inherited as an autosomal-recessive trait. The true incidence is not known, but the peripheral form appears to be approximately 10 times as common in African Americans as in other groups. The epimerase gene is on chromosome 1. A number of mutations have been identified and characterised. The V94M mutation was present in a homozygous form in all of the patients tested with the severe phenotype [40], whereas other mutations are associated with the intermediate or asymptomatic phenotype [39, 41].

7.2.4 Diagnostic Tests

GALE deficiency should be suspected when red cell galactose-1-phosphate is increased while GALT is normal. Newborn screening will give an abnormal result if defined by a raised total blood galactose level with normal GALT activity. Diagnosis is confirmed by the assay of epimerase in erythrocytes. Heterozygous parents have reduced epimerase activity, a finding that can help in the evaluation. Further studies of GALE activity in transformed lymphoblasts and red cell Gal-I-P while on and off dietary galactose may help characterise the disorder further [39]. In those families with the severe form of GALE deficiency, DNA analysis for the V94M mutation has been the most rapid method of determining whether infants at risk are affected or not.

7.2.5 Treatment and Prognosis

Newborns at risk of the severe form of GALE deficiency must have lactose excluded from their diet until the re-

sults of diagnostic tests are available. It is unclear whether infants diagnosed with this form of the disorder require a small amount of exogenous galactose for synthesis of galactoproteins and galactolipids. As with GALT deficiency, red cell Gal-1-P levels have not been particularly helpful for long-term monitoring; concentrations have remained raised on treatment, but to a lesser degree than those seen in patients with GALT deficiency. The oldest patients homozygous for V94M mutation are now in their 3rd decade; they have not shown evidence of progressive disease.

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

7.3 Galactokinase Deficiency

7.3.1 Clinical Presentation

Cataracts are the only consistent manifestation of untreated galactokinase (GALK) deficiency, though pseudotumour cerebri has been described [42]. Liver, kidney and brain damage are not seen.

7.3.2 Metabolic Derangement

Persons with GALK deficiency lack the ability to phosphorylate galactose (◘ Fig. 7.1) and consequently accumulate galactose and galactitol, the latter being formed from galactose by aldose reductase. Individuals on a normal galactose intake have high levels of galactose and galactitol in blood and urine. Galactitol also accumulates in the lens, causing osmotic swelling of lens fibres and denaturation of proteins. Galactose-1-phosphate concentrations are not raised.

7.3.3 Genetics

The mode of inheritance is autosomal recessive. In most parts of Europe and in the USA and Japan, the birth incidence is in the order of 1 in 150,000 to 1 million. It is higher in the Balkan countries [43], the former Yugoslavia, Rumania and Bulgaria. In Roma people, the birth incidence has been calculated as 1 in 2,500.

Two genes have been reported to encode galactokinase: *GK1* on chromosome 17q24 and *GK2* on chromosome 15. *GK1* mutations cause GALK deficiency, and many have now been described. The *GK1* P28T mutation was identified as the founder mutation responsible for galactokinase deficiency in Roma and in immigrants from Bosnia in Berlin [44]. The *GK2* gene product is primarily a *N*-acetylgalactosamine kinase, and although it does have some galactokinase activity this is insufficient to prevent the accumulation of galactose and galactitol in those with *GK1* mutations.

7.3.4 Diagnostic Tests

Provided they have been fed breast milk or a lactose-containing formula prior to the test, newborns with the defect are discovered by NBS methods for detecting elevated blood galactose. If they have been fed glucose-containing fluid the result of the screening test could be false negative. Any chance finding of a reducing substance in urine, especially in children or adults with nuclear cataracts, calls for identification of the excreted substance. In addition to galactose, galactitol and glucose may be found. Every person with nuclear cataracts ought to be examined for GALK deficiency. The final diagnosis is made by assaying GALK activity in heparinised whole blood, red cell lysates, liver or fibroblasts. Heterozygotes have intermediate activity in erythrocytes.

7.3.5 Treatment and Prognosis

Treatment may be limited to the elimination of milk from the diet. Minor sources of galactose, such as milk products, green vegetables, legumes and drugs in tablet form, can probably be disregarded, since it can be assumed that the small amounts of ingested galactose are either metabolised or excreted before significant amounts of galactitol can be formed. Once a patient is on a galactose-restricted diet urinary levels of galactitol return to normal. When the diagnosis is made rapidly and treatment begun promptly, i.e. during the first 2-3weeks of life, cataracts can clear. When treatment is late and cataracts too dense, they will not clear completely (or at all) and must be removed surgically. In patients who have had their lenses removed, recurring cataracts may appear, originating from remnants of the posterior lens capsule. This can be avoided by continuing the diet.

As in carriers with GALT deficiency, the speculation that heterozygosity for GALK deficiency predisposes to the formation of presenile cataracts remains unproven [45]. It has been suggested that heterozygotes restrict their milk intake [46], though scientific proof of the merits of this measure is lacking.

7.4 Fanconi-Bickel Syndrome

This is a recessively inherited disorder of glucose and galactose transport; it is due to deficiency of glucose transporter 2 (GLUT2) and is rare. 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 11.

7.5 Portosystemic Venous Shunting and Hepatic Arteriovenous Malformations

Portosystemic bypass of splanchnic blood via ductus venosus or intrahepatic shunts causes alimentary hypergalactosaemia, which is discovered during metabolic NBS [47].

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Disorders of the Pentose Phosphate Pathway

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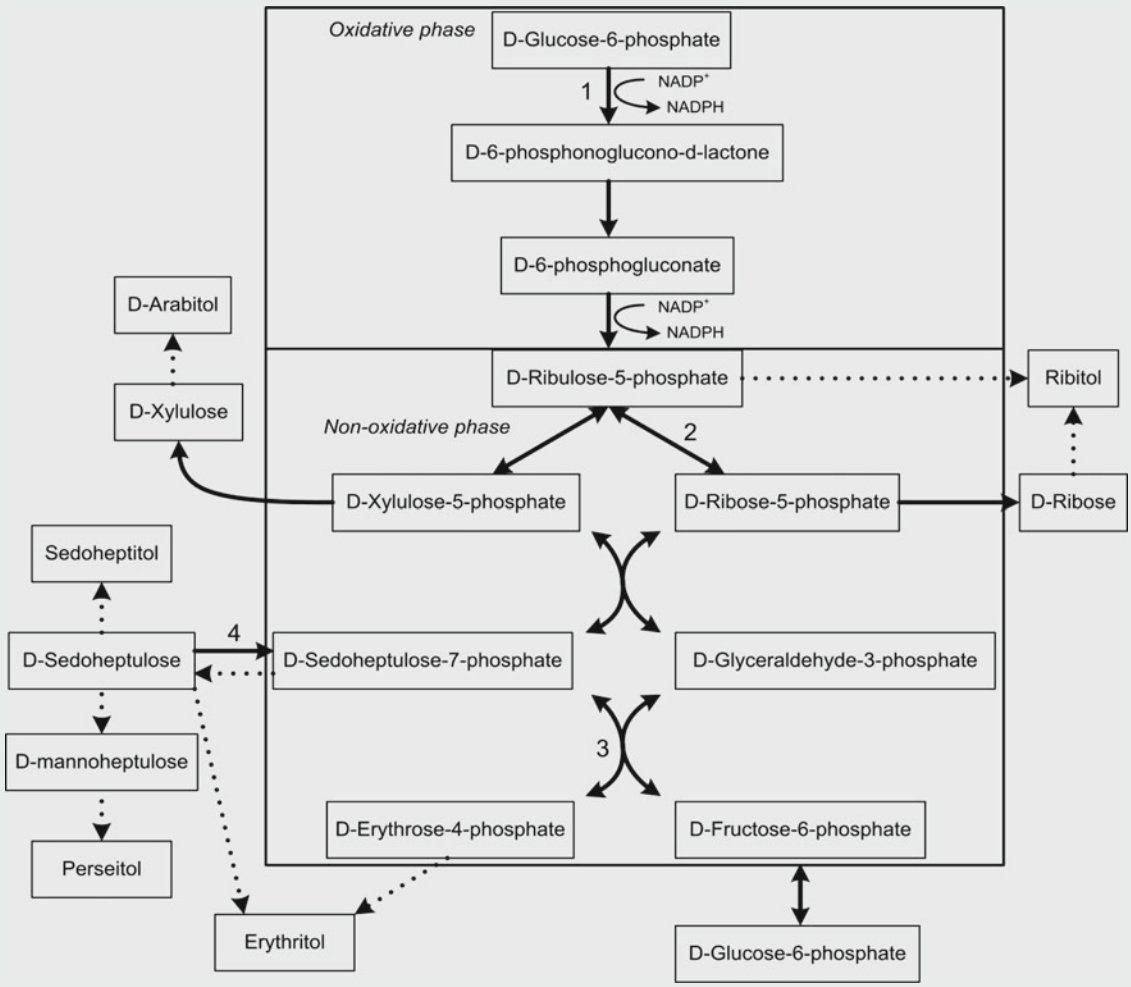
J.-M. Saudubray et. al (Eds.), *Inborn Metabolic Diseases,* DOI 10.1007/978-3-642-15720-2_8,

The Pentose Phosphate Pathway

The pentose phosphate pathway (PPP; ◘ Fig. 8.1) consists of two distinct parts, which fulfil two specific roles: the first part, an oxidative, nonreversible pathway, allows reduction of nicotinamide adenine dinucleotide phosphate ($NADP^+$) while converting glucose-6-phosphate to a pentose-phosphate and CO_2, and the second part, a nonoxidative, reversible pathway, produces ribose for nucleotide and nucleic acid synthesis and connects pentose phosphates to glycolytic intermediates. To date, three inborn errors in the PPP have been described.



◘ **Fig. 8.1.** The pentose phosphate pathway. The conversion of the sugar phosphates into their corresponding sugars and polyols (*dotted arrows*) has not been proven in humans. *NADP*, nicotinamide adenine dinucleotide phosphate; *NADPH*, reduced form; 1, glucose-6-phosphate dehydrogenase; 2, ribose-5-phosphate isomerase; 3, transaldolase; 4, sedoheptulokinase

Three inborn errors in the pentose phosphate pathway (PPP) are known.

Glucose-6-phosphate dehydrogenase deficiency is a defect in the first, irreversible step of the pathway. As a consequence, NADPH production is decreased, making erythrocytes vulnerable to oxidative stress. Drug- and fava bean-induced haemolytic anaemia is the main presenting symptom of this defect. G6PD deficiency is an X-linked disorder. As this is a haematological disorder it is not discussed further.

Deficiency of ribose-5-phosphate isomerase has been described in one patient, who presented with developmental delay and a slowly progressive leukoencephalopathy.

Transaldolase deficiency has been diagnosed in ten unrelated families. All patients presented in the neonatal or antenatal period with hepatosplenomegaly, liver function problems, hepatic fibrosis and haemolytic anaemia.

Moreover, the most common, 57-kb deletion in nephropathic cystinosis patients, has been shown also to cause deficiency of **sedoheptulokinase**, resulting in urinary accumulation of sedoheptulose [1] (► Chapter 43).

Essential pentosuria is the result of a partial deficiency of L-xylulose reductase (xylitol dehydrogenase) and affects the related glucuronic acid pathway. Whereas the PPP involves D-stereoisomers, glucuronic acid gives rise to L-xylulose, which is subsequently converted into xylitol and D-xylulose. Affected individuals excrete large amounts of L-xylulose in urine. Pentosuria occurs almost exclusively in Jewish people. This is a benign disorder and is not discussed further here.

8.1 Ribose-5-Phosphate Isomerase Deficiency

8.1.1 Clinical Presentation

The one patient recorded with ribose-5-phosphate isomerase (RPI) deficiency, a boy, was born after an uncomplicated pregnancy as the only child of healthy, unrelated parents [2, 3]. He had psychomotor retardation from early in life and developed epilepsy at the age of 4 years. From the age of 7 years a slow neurological regression occurred, with prominent cerebellar ataxia, some spasticity, optic atrophy and a mild sensorimotor neuropathy. Neither organomegaly nor dysfunction of the internal organs was present. His growth parameters were normal. He is now in his twenties. 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, or the sugar and polyol region of the spectrum, which were identified as representing ribitol and D-arabitol.

8.1.2 Metabolic Derangement

RPI 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 xylulose-5-phosphate. The concentrations of ribitol and arabitol displayed a steep descending brain/CSF/plasma gradient.

8.1.3 Genetics

The human *RPIA* gene 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 at codon 181 and a predicted truncated protein of 196 amino acids, and a missense mutation c.182C>T, resulting in an ala-to-val substitution (p.A61V). The finding of two mutant alleles in the patient and apparently healthy parents suggests autosomal recessive inheritance.

8.1.4 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 elevated (more than 10 times the upper limit of the reference ranges). 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, which correspond to arabitol and ribitol.

The diagnosis can be confirmed by an enzyme assay in fibroblasts or lymphoblasts, and by sequence analysis of the *RPIA* gene.

8.1.5 Treatment and Prognosis

Therapeutic options for RPI deficiency have not yet been identified. The prognosis is unclear.

8.2 Transaldolase Deficiency

8.2.1 Clinical Presentation

Transaldolase (TALDO) deficiency was first described in 2001 [4] in a single patient presenting with cirrhosis, with nine additional patients reported from five different families [4-9].

The patients were each born to consanguineous couples native to Turkey, the United Arabic Emirates,

Pakistan or Poland, suggesting rare alleles in the human population.

Wide phenotypic variability has been reported in TALDO-deficient patients (◘ Table 8.1) [4-7, 9, 10]. Most patients display the first symptoms of the disease in the neonatal or antenatal period, when intrauterine growth retardation, oligohydramnios and hydrops fetalis have been described, leading to a medical termination of pregnancy in the case of a 28-week fetus presenting also with a polymalformative syndrome [6]. Neonates present with hepatosplenomegaly, bleeding, abnormal liver function tests, cholestatic jaundice and elevated liver enzymes. Hepatic fibrosis and cirrhosis (in older patients) are the pathological liver hallmarks. Haemolytic anaemia is also a constant feature found in all patients.

Most patients showed dysmorphic features (antimongoloid slant, low-set ears and cutis laxa, hypertrichosis), neonatal oedema and congenital heart defects (septal defects, cardiomyopathy, tetralogy of Fallot).

Renal manifestations (tubulopathy, renal failure, nephrocalcinosis) and endocrine disorders have frequently been reported, leading to intermittent hypoglycaemia, and testicular or ovarian insufficiency leading to cryptorchidism, clitoris enlargement, intermittent hypoglycaemia 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 (three 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.

8.2.2 Metabolic Derangement

TALDO 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 derived from the pathway intermediates and seven-carbon sugars from sedoheptulose-7-phosphate. In a majority of TALDO-deficient patients elevated urinary concentration of the citric acid cycle intermediates 2-oxoglutaric acid and fumaric acid were detected, indicating a possible disturbed mitochondrial metabolism in TALDO deficiency [11].

8.2.3 Genetics

The human *TALDO* gene (*TALDO1*) is 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 the *TALDO1* gene include missense mutations, deletions and a duplication.

◘ **Table 8.1.** Principal clinical manifestations in reported patients with TALDO deficiency

Patient no. [ref.]	1 [4]	2 [5]	3 [6]	4 [6]	5 [6]	6 [6]	7 [8]	8 [10]	9 [9]	10 [9]
Dysmorphism or cutis laxa	-	+	+	+	+	+	+	+	-	-
Consanguinity	+	+	+	+	+	+	+	+	+	+
IUGR	+	-	+	+	+	+	+	+	+	-
Neonatal oedema/oligoamnios	-	+	-	++	-	+	+	-	+	-
Liver – function – fibrosis/cirrhosis	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Anaemia	+	+	+	-	+	+	+	-	+	+
Cardiac	+	+	+	+	+	+	+	-	-	-
Renal	+	+	+	?	+	+	+	-	-	-
Endocrine	-	?	+	?	-	+	-	-	-	-
Psychomotor development	n†	?†	n†	?†	n	n	Mild delay	Mild delay	n	n

IUGR, intrauterine growth retardation; +, presence; -, absence; ?, unknown; †, deceased patient

8.2.4 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 by liquid chromatography-tandem mass spectrometry [12, 13]. 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 [14]. 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 the *TALDO* gene.

Prenatal diagnosis is possible by sequence analysis of the *TALDO* gene in chorionic villi and amniocytes. In amniotic fluid from an affected fetus increased concentrations of sedoheptulose and ribitol were detected [15]. Prenatal diagnosis may therefore also be possible by measuring sedoheptulose and ribitol in amniotic fluid supernatant.

8.2.5 Treatment and Prognosis

Prognosis is variable in patients with TALDO deficiency. For most patients the outcome seems to be correlated to the severity of the liver impairment. Three patients died of acute liver failure at the onset of the disease (2/3) or of chronic liver failure-related complications (1/3). However, a few patients with an initially severe liver disease and fibrosis are currently stable. In one all liver symptoms, including hepatomegaly, resolved (patient 5 in Table 8.1) [6]. Given the variability in phenotype and outcome, TALDO deficiency could be considered in adults with cirrhosis of unknown aetiology, even though no adult patients have been reported so far.

There is no specific treatment for TALDO deficiency. Liver manifestations may receive symptomatic treatment or require liver transplantation. Hormone disturbances 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 potent antioxidant drugs such as *N*-acetylcysteine [16].

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Disorders of Fructose Metabolism

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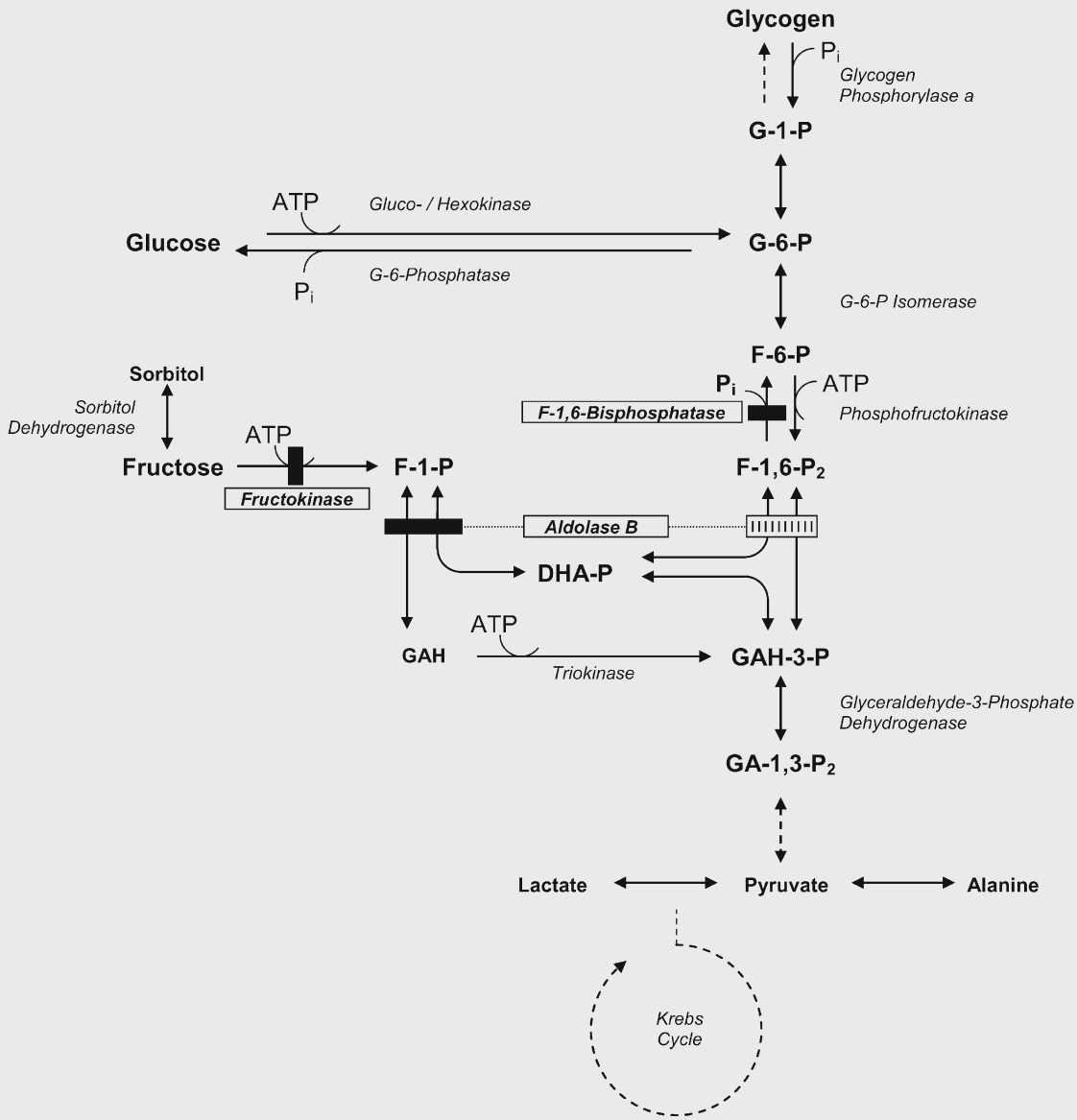
Fructose Metabolism

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 to fructose in the liver by sorbitol dehydrogenase (◘ Fig. 9.1). 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 also intervenes in the glycolytic-gluconeogenic pathway (◘ Fig. 9.1, right-hand part of the scheme).



◘ **Fig. 9.1.** 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 depicted in ◘ Fig. 9.1. **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 may 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 it is 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 can also be life threatening.

9.1 Essential Fructosuria

9.1.1 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. 9.1).

9.1.2 Metabolic Derangement

In essential fructosuria, ingested fructose is partly (10-20%) excreted unchanged in the urine and the rest is slowly metabolised by an alternative pathway, namely conversion to fructose-6-phosphate by hexokinase in adipose tissue and muscle.

9.1.3 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, it may be more prevalent than reported. This may become even more the case as clinical laboratories continue to abandon tests for reducing substances in urine in favour of specific glucose tests.

The *KHK* gene is located on chromosome 2p23.3-23.2. Tissue-specific alternative splicing results in two isoforms, ketohexokinase A, which is widely distributed in most fetal and adult organs but with no clear physiological role, and ketohexokinase C, which is expressed in adult liver, kidney and small intestine and which is affected in essential fructosuria [2]. To date, only two mutations of the *KHK* gene, 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].

9.1.4 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 and sucrose intake and, thus, is inconstant. Fructose tolerance tests (▶ Section 9.2) do not provoke an increase in blood glucose, as in normal subjects, or the hypoglycaemia or other changes that occur in HFI and FBPase deficiency; nor are metabolic changes in liver detectable by ^{31}P-magnetic resonance spectroscopy (MRS) [5].

9.1.5 Treatment and Prognosis

Dietary treatment is not indicated, and the prognosis is excellent.

9.2 Hereditary Fructose Intolerance

9.2.1 Clinical Presentation

Infants, children and adults with hereditary fructose intolerance (HFI) are perfectly healthy and asymptomatic 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/saccharose-free infant formula, the first symptoms appear with the intake of fruits and vegetables [6, 7]. 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 a 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 easily be 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 to 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 [8]. Some adult cases were diagnosed after the patients developed life-threatening reactions to infusions containing fructose, sorbitol or invert sugar (a mixture of glucose and fructose obtained by hydrolysis of sucrose) when these i.v. solutions were still in use [9]. Because approximately half of all adults with HFI are free of caries, the diagnosis has also been made by dentists. Although several hundred patients with HFI have been identified since its recognition as an inborn error of metabolism in 1957 [6], these observations indicate that affected subjects may remain undiagnosed and still have a normal life span.

9.2.2 Metabolic Derangement

HFI is caused by deficiency of the second enzyme of the fructose pathway, aldolase B (fructose-1,6-bisphosphate aldolase) (◘ Fig. 9.1), 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 the trapping of phosphate. This has two major effects [10]: (1) inhibition of glucose production by blockage of gluconeogenesis (e.g. by inhibition of aldolase A) and of glycogenolysis (e.g. by inhibition of glycogen phosphorylase a), which induces a rapid drop in blood glucose, and (2) overutilisation and diminished regeneration of ATP. This depletion of ATP results in an increased production of uric acid and 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 [11] (► Chapter 41).

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 i.v. administration of fructose to normal subjects also induces the metabolic derangements described above (including the drop in ATP and inorganic phosphate and the rise in urate and magnesium) to an equivalent extent, although they are more transient than in patients with HFI, as demonstrated by ^{31}P-MRS [5]. In normal subjects, i.v. 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 [12].

9.2.3 Genetics

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. The human gene for aldolase B (*ALDOB*) has been mapped to chromosome 9q22.3. At present, according to different databases, approximately 50 causative mutations of the *ALDOB* gene 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 [13] and have been detected in certain populations with a frequency among mutated alleles of up to 70%, 23% and 10%, respectively. Some mutations may be found particularly in certain ethnic groups, such as p.N335K in patients from former Yugoslavia, c.360-363-delCAAA in patients from Sicily, or mutations upstream of the protein-coding region of the *ALDOB* gene in Hispanics or African-Americans [14]. The effect of a number of mutations on the three-dimensional structure of the human aldolase B protein has been determined [15].

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/hfidb/DistribTable.htm), a noninvasive diagnostic approach using molecular genetic methods has been advocated, and specific methods for the

[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.

rapid concomitant detection of these frequent *ALDOB* mutations have been published[1] [16, 17].

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 [16].

9.2.4 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, mellituria, generalised hyperaminoaciduria, hyperphosphaturia, hypophosphataemia, metabolic acidosis).

A cornerstone of the diagnosis of HFI is a careful nutritional history, with special emphasis on the time of weaning, when fruits and vegetables were introduced [1, 7, 18]. 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 noninvasive approach and has the advantage over enzymatic measurement in liver tissue that it eliminates the complication of secondarily lowered aldolase 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%) [18], although residual activities as high as 30% of normal have been reported [9]. There is also a distinct (but less marked) reduction in the activity of aldolase towards 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-P, which is approximately 1 in control liver, is increased to 2-∞ in HFI patients [18]. 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 discouraged. For post-mortem 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 body weight) is injected i.v. as a 20% solution within 2 min. Blood samples are taken at 0, (2), 5, 10, 15, 30, 45, 60 and 90 min for determination of glucose and phosphate. In normal subjects, blood glucose increases by 0-40%, with minimal changes or none at all in phosphate [18]. In HFI patients, glucose and phosphate decrease within 10-20 min. 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 i.v. glucose administration. Oral fructose tolerance tests are not recommended, because they provoke more ill-effects and are less reliable [18].

9.2.5 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, intrauterine infections, glycogen and other storage disorders, ornithine transcarbamoylase 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 [19], a condition caused by defective fructose transport in the small intestine but whose metabolic basis 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 definitively excluded before such a tolerance test is performed, as otherwise deleterious effects may occur [20]. In sucrase-isomaltase deficiency, the ingestion of sucrose results in bloating, abdominal cramps and fermentative osmotic diarrhoea; free fructose, however, is well tolerated.

9.2.6 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 connection, it is deplorable that European Union regulations allow infant formulae to contain up to 20% of their total carbohydrate content as sucrose [21].

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 dietitian should be consulted for discussion of 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). Substitution of vitamins, especially ascorbic acid and folates, in the form of a multivitamin preparation should be prescribed to make up for those patients are lacking through their inability to have fruits and vegetables included in their diet.

After institution of a fructose-free diet most abnormalities disappear rapidly, except for hepatomegaly, which may persist for months or even years [22]. 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 to 250 mg/kg/day, as against 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 once a stricter diet is instituted [8]. It must also 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. in hereditary fructose intolerance, has been suggested for dietary monitoring [11]; however, the sensitivity of this promising procedure has not been definitively evaluated. Needless to say, 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 on fructose ingestion by mistake, and that is why HFI, if present, should be reported on any hospital admission by means of an emergency card.

The prognosis is excellent, with normal growth, intelligence and life span.

9.3 Fructose-1,6-Bisphosphatase Deficiency

9.3.1 Clinical Presentation

In about half of all cases, fructose-1,6-bisphosphatase (FBPase) deficiency presents in the first 1-4 days of life with severe hyperventilation caused by profound lactic acidosis and marked hypoglycaemia. Later on, episodes of irritability, somnolescence or coma, apnoeic spells, dyspnoea and tachycardia, muscular hypotonia and moderate hepatomegaly may occur. As reported in the first patient described [23], 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 body weight in one dose), especially after a period of fasting. FBPase deficiency may be life threatening and, as in HFI, i.v. administration of fructose is contraindicated and may lead to death. 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 [24].

Most affected children experience a number of acute attacks before the diagnosis is made. Once diagnosis is established and treatment begins the course is favourable, and pregnancies have been documented [25, 26].

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 30), hyperalaninaemia and glucagon-resistant hypoglycaemia. Hyperketonaemia may be found, but in several patients ketosis has been reported to be moderate or absent (► below) [27]. 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.

9.3.2 Metabolic Derangement

Deficiency of hepatic FBPase, a key enzyme in gluconeogenesis, impairs the formation of glucose from all gluconeogenic precursors, including dietary fructose (◘ Fig. 9.1). 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 [28]. Thus, hypoglycaemia is likely to occur when glycogen reserves are limited (as in newborns) or exhausted (as when fasting). The defect moreover provokes accumulation of the gluconeogenic substrates lactate, pyruvate, alanine and glycerol. The lactate/pyruvate ratio is usually increased, owing to secondary impairment of the conversion of 1,3-bisphosphoglycerate to glyceraldehyde-3-phosphate, resulting in accumulation of reduced nicotinamide adenine dinucleotide, the other substrate of glyceraldehyde-3-phosphate dehydrogenase (not shown in ◘ Fig. 9.1). Attention has been drawn to the fact that hyperketonaemia and ketonuria, which usually accompany hypoglycaemia, may be absent in some patients with FBPase deficiency [27]. 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 body weight per day, when given regularly distributed over the day and, in contrast to subjects with HFI, they thrive on such a diet. Nevertheless, loading tests with 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, FBPase deficiency still allows F-1-P to be converted into lactate. ^{31}P-MRS of the liver following i.v. administration of fructose (200 mg/kg body weight) 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 relative to healthy controls [5].

9.3.3 Genetics

FBPase deficiency is an autosomal recessive disorder. Its frequency seems to be much lower than that of HFI; a first estimation of 1:350,000 has been reported for the Netherlands [24]. 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 from the liver isoform and is not affected in patients with FBPase deficiency. Only the liver-type isoform gene (*FBP1*) has been cloned and characterised to date. It has been localised to chromosome 9q22.2–q22.3. *FBP1* mutations were first reported in 1995 [29], and to date approximately 20 different mutations have been published. Among them are single nucleotide exchanges, small deletions and insertions and one gross deletion. All regions of the gene may be affected and, with the exception of the c.961insG mutation, which has been reported to be responsible for 46% of mutated alleles in Japan [30] but only 14% in Central Europe [31], no single mutation is particularly frequent.

There are several FBPase-deficient patients in whom no mutation of the coding region of *FBP1* has been found. Therefore, it has been supposed that these patients carry mutations within the promoter region of *FBP1* or, more hypothetically, in the gene for the bifunctional enzyme that controls the concentration of fructose-2,6-bisphosphate, the main physiological regulator of FBPase [32].

9.3.4 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 clinical and laboratory findings, determination of enzymatic activity in a liver biopsy should be undertaken. In symptomatic cases, the residual activity may vary from zero to 30% of normal, indicating genetic heterogeneity of the disorder. Obligate heterozygotes have intermediate activity. Diagnosis is unreliable in mixed leukocytes [33], but seems to be reliable in isolated and stimulated monocytes [26]. Cultured skin fibroblasts, amniotic fluid cells and chorionic villi do not exhibit FBPase activity.

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.

9.3.5 Differential Diagnosis

Other disturbances in gluconeogenesis and pyruvate oxidation have to be considered, including (1) pyruvate dehydrogenase deficiency characterised by a low lactate/pyruvate ratio, absence of hypoglycaemia and aggravation of lactic acidosis by glucose infusion; (2) pyruvate carboxylase deficiency; (3) respiratory chain disorders; (4) glycogenosis type Ia and type I *non*-a presenting with the same metabolic profile (fasting hypoglycaemia and lactic acidosis) and hepatonephromegaly, hyperlipidaemia and hyperuricaemia; and (5) fatty acid oxidation defects presenting with fasting hypoketotic hypoglycaemia and hyperlactataemia.

9.3.6 Treatment and Prognosis

Whenever FBPase deficiency is suspected, adequate amounts of i.v. or oral glucose should be given. The acute, life-threatening episodes should be treated with an i.v. bolus of 20% glucose followed by a continuous infusion of glucose at high rates (10-12 mg/kg/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, note that the infusion of glycerol (which may even contain additional fructose), as frequently practised in patients with brain oedema and hypoglycaemia in Japan, is extremely dangerous unless FBPase deficiency has been ruled out [34].

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 and both 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 [25, 26]. Many patients, however, become obese because their concerned parents overfeed them and they continue these eating habits when they are older. Under carefully observed conditions, a hypocaloric diet can lead to a considerable weight loss in obese patients without the development of lactic acidosis and hypoglycaemia (B. Steinmann, personal observations).

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Persistent Hyperinsulinaemic Hypoglycaemia

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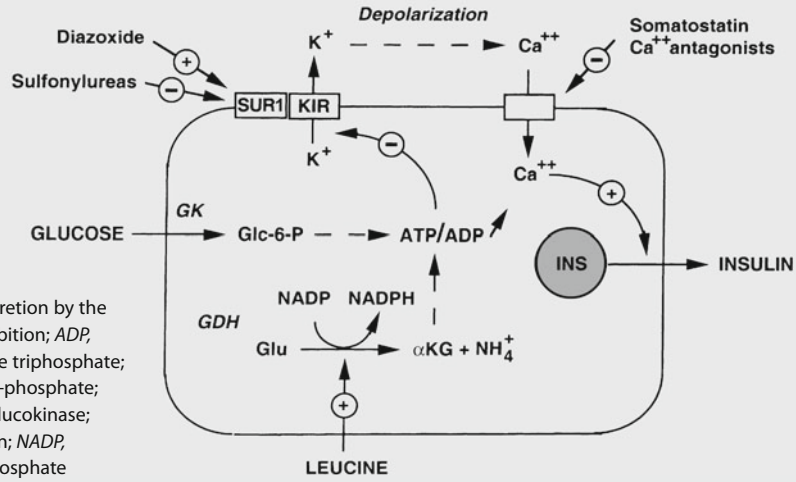
Glucose-induced Insulin Secretion and Its Modulation

After glucose enters the pancreatic β-cell it is phosphorylated to glucose-6-phosphate by glucokinase. This enzyme, with a K_m for glucose close to its concentration in blood, functions as a glucose sensor. A small change in blood glucose increases the rate of glucose metabolism, the generation of ATP by the glycolytic pathway and the concentration of ATP relative to ADP. The increase in ATP results in closure of K^+ channels (composed of two subunits, a K^+-ATP channel [KIR] and the sulfonylurea receptor [SUR1]), membrane depolarisation, opening of voltage-sensitive Ca^{2+} channels, influx of extracellular Ca^{2+}, and stimulation of insulin secretion by exocytosis from storage granules.

Leucine, a potent enhancer of insulin secretion, acts by allosteric stimulation of glutamate dehydrogenase (GDH). This results in an increase in the formation of α-ketoglutarate (an intermediate of the Krebs cycle) and, hence, in elevation of ATP. Diazoxide inhibits insulin secretion by activating (opening) SUR1, whereas sulfonylureas, such as tolbutamide, stimulate insulin secretion by closing SUR1. Somatostatin and Ca^{2+} antagonists inhibit insulin secretion by decreasing Ca^{2+} influx. Other components (not shown in ◘ Fig. 10.1) that play a role in the regulation of insulin secretion are short-chain L-3-hydroxyacyl-CoA dehydrogenase (SCHAD), the monocarboxylate transporter MCT1, the mitochondrial uncoupling protein UCP2, and transcription factors such as HNF1-α.



◘ **Fig. 10.1.** Mechanisms of insulin secretion by the pancreatic β-cell. +, stimulation; -, inhibition; *ADP,* adenosine diphosphate; *ATP,* adenosine triphosphate; *α-KG,* α-ketoglutarate; *G-6-P,* glucose-6-phosphate; *GDH,* glutamate dehydrogenase; *GK,* glucokinase; *Glc,* glucose; *Glu,* glutamate; *INS,* insulin; *NADP,* nicotinamide adenine dinucleotide phosphate

Hyperinsulinism can present throughout childhood but is most common in infancy. Persistent hyperinsulinaemic hypoglycaemia of infancy (HI) is the most important cause of hypoglycaemia in early infancy. The excessive secretion of insulin is responsible for profound hypoglycaemia and requires aggressive treatment to prevent severe and irreversible brain damage. Onset can be in the neonatal period or later, with the severity of hypoglycaemia decreasing with age. HI is a heterogeneous disorder with two histopathological lesions, diffuse (DiHI) and focal (FoHI), which are clinically indistinguishable. FoHI is sporadic and characterised by somatic islet cell hyperplasia. DiHI corresponds to a functional abnormality of insulin secretion in the whole pancreas and is most often recessive, although rare dominant forms can occur, usually outside the newborn period. In addition to these two well-defined forms, some patients have an atypical HI with mosaicism of their pancreatic cells rather than the typical focal or diffuse hyperplasia. Differentiation between focal and diffuse lesions is important, because the therapeutic approach and genetic counselling differ radically. Injection of [^{18}F]fluoro-l-DOPA, followed by positron emission tomography coupled with computed tomography (PET-CT) is a recent test that can distinguish between FoHI and DiHI. A combination of glucose and glucagon is started as an emergency treatment as soon as a tentative diagnosis of HI is made. This is followed by diazoxide and other medication. Patients who are resistant to medical and dietary treatment require pancreatectomy; FoHI can be definitively cured by a limited pancreatectomy, but DiHI requires a subtotal pancreatectomy, following which there is a high risk of diabetes mellitus. Persistent hyperinsulinism in older children is most commonly caused by pancreatic adenoma.

10.1 Clinical Presentation

Severe hypoglycaemia, with its high risk of seizures and brain damage, is the major feature of hyperinsulinism (HI) [1, 2]. The presentation varies according to the age of onset. In the neonatal period hypoglycaemia is severe, occurs within 72 h after birth, and is manifest in half of the patients by seizures. The majority of affected newborns are macrosomic at birth, with a mean birthweight of 3.7 kg, and approximately 30% are delivered by cesarean section [1]. Other symptoms are abnormal movements, tremulousness, hypotonia, cyanosis, hypothermia and life-threatening events. In some cases, hypoglycaemia is discovered by routine measurement of blood glucose. The concentration at the time of the first symptoms is often extremely low (<1 mmol/l). Hypoglycaemia is persistent, occurring in both the fed and the fasting state. The rate of i.v. glucose administration required to maintain plasma glucose above 3 mmol/l is high, with a mean of 17 mg/kg/min. The blood glucose concentration can be increased by 2-3 mmol/l by s.c. or i.m. administration of glucagon (0.5 mg). Mild hepatomegaly is frequently found.

Hypoglycaemia from HI presenting later in infancy (1-12 months of age) has a similar clinical presentation but usually requires a lower rate of i.v. glucose to maintain a normal blood glucose [3]. Macrosomy at birth is common, and seizures occur in approximately 50% of patients.

Hypoglycaemia from HI first presenting in childhood usually occurs between 4 and 13 years of age and is highly suggestive of a pancreatic adenoma, the histology of which is different from that of the focal lesion. The rate of oral or i.v. glucose required to maintain normal plasma glucose is lower, and not all children require continuous glucose administration. Because hypoglycaemia is better tolerated, the diagnosis is often delayed.

In all types of HI, facial dysmorphism with high forehead, large and bulbous nose with short columella, smooth philtrum and thin upper lip is frequently observed [4]. It should be noted that HI can be part of syndromic His, such as Usher syndrome type Ic or congenital disorders of glycosylation [5]. A few patients with Beckwith-Wiedemann, Perlman or Sotos syndrome, and Kabuki syndrome have also been described with HI. Fabricated or induced illness (FII: the so-called Münchausen by proxy syndrome) should be included in the differential diagnosis. Epilepsy and mental retardation appear to be frequent in patients with HI associated with hyperammonaemia (HI/HA), and are not explained by hypoglycaemia [6].

In exercise-induced HI (EIHI), a novel autosomal dominant form of HI, the patients suffer from hypoglycaemic symptoms only when performing strenuous physical exercise [7].

Postprandial, as well as fasting, hyperinsulinaemic hypoglycaemia associated with resistance to insulin has also been observed in a syndrome of dominant HI in which the insulin receptor gene is implicated [8]. Relatives of patients with HNF4-α mutations present with a transient neonatal HI, before going on to develop maturity-onset diabetes (MODY) [9]. Finally, transient HI can also result from neonatal or fetal distress.

10.2 Metabolic Derangement

Hyperinsulinaemic hypoglycaemia is due to either focal or general insulin hypersecretion by the pancreas [1]. Insulin decreases plasma glucose both by inhibiting hepatic glucose release from glycogen and gluconeogenesis and by increasing glucose uptake in muscle and fat.

HI is a heterogeneous disorder which can be caused by various defects in the regulation of insulin secretion by the pancreatic β-cell (◘ Fig. 10.1) [10]. These include (1) channelopathies affecting either the SUR1 [11, 12] or the KIR channel [13, 14]; (2) enzyme defects involving glucokinase (GK) [15], glutamate dehydrogenase (GDH) [16], or short-chain l-3-hydroxyacyl-CoA dehydrogenase (SCHAD) [17]; (3) defects of the monocarboxylate transporter 1 (MCT1) [18] and the mitochondrial uncoupling protein 2 (UCP2) [19]; and (4) modifications of insulin secretion implicating the insulin receptor [8] and, more recently, HNF4-α, a transcription factor involved in MODY [9].

10.3 Genetics

The estimated incidence of HI is 1 in 50,000 live births, but in countries with substantial consanguinity, such as Saudi Arabia, it may be as high as 1 in 2500 [20]. The two main histological forms of HI, focal and diffuse islet cell hyperplasia, correspond to distinct molecular entities.

▪ Focal Islet Cell Hyperplasia

Focal islet-cell hyperplasia is associated with isodisomy of paternally inherited mutations of either the sulfonylurea-receptor (*ABCC8* encoding SUR1) or the inward rectifying potassium channel gene (*KCNJ11* encoding Kir6.2) on chromosome 11p15, with a loss of the maternal allele in the hyperplastic islets [21-23]. The focal hyperplasia probably is a sporadic event, as indicated by the somatic molecular abnormality in the pancreas and by the observation of discordant identical twins [21].

- **Diffuse Islet Cell Hyperplasia**

Diffuse islet cell hyperplasia is associated with many inherited disorders transmitted with various modes of inheritance:

- Recessive *ABCC8* mutations (encoding SUR1), and more rarely recessive *KCNJ11* mutations (encoding Kir6.2), are responsible for the majority of cases of *diffuse* and severe neonatal HI resistant to medical treatment [11-14]. Identification of these mutations should eventually allow prenatal diagnosis.
- Another recessively inherited HI involves short-chain l-3-hydroxyacyl-CoA dehydrogenase (SCHAD, a mitochondrial fatty acid oxidation enzyme encoded by the *HAD*H gene, which is specifically overexpressed in the pancreatic β-cells). In this defect, HI seems less severe and is sensitive to medical treatment, and there are no apparent liver, cardiac or muscle clinical signs of fatty acid oxidation defect [17]. The diagnosis cannot be suspected on a clinical basis, relying solely on the finding of increased 3-OH-butyryl-carnitine in plasma, and is confirmed by enzyme assay in fibroblasts and DNA analysis. Recent animal studies have shown that SCHAD deficiency causes hyperinsulinism by activating GDH, which is normally inhibited by SCHAD [22].
- Dominant *ABCC8* mutations (SUR1) are responsible for less severe HI which occurs in the 1st year of life and is sensitive to diazoxide [23, 24].
- Dominantly expressed missense mutations of *GLUD1,* the gene encoding the mitochondrial matrix enzyme, GDH, cause hyperinsulism/hyperammonaemia (HI/HA) syndrome, the second most common form of HI [16]. In this syndrome HI may be severe but responds to medical treatment. Hyperammonaemia is mild to moderate (80-250 μmol/l), is not related to the protein intake, and is not accompanied by any significant disturbance in the amino acid profile. Several mutations, mostly located in the allosteric site, result in a gain of function by way of loss of sensitivity of the enzyme to its allosteric inhibitor, GTP, leading to inappropriate production of αKG and ammonia.
- Dominantly expressed glucokinase (*GK*) mutations are a rare cause of HI. They result in a gain of function by increasing the affinity of *GK* for glucose, leading to inappropriate insulin secretion [15, 25, 26]. These mutations are remote from the glucose-binding site and suggest an allosteric regulation defect.
- Dominantly expressed mutations in the *SLC16A1* gene encoding the monocarboxylate transporter 1 have been more recently described, causing exercise-induced HI (EI/HI syndrome) [18].
- The mitochondrial uncoupling protein 2 (UCP2) [19] and the hepatocyte nuclear factor 4A (HNF4A) [9] have recently been shown to be involved in HI.

Mosaic islet cell hyperplasia, associated with an atypical presentation of less severe HI, still remains poorly understood [27].

The aetiology of pancreatic adenomas is unknown except for MEN1 syndrome, in which menine protein deficiency is caused by a dominant mutation of the *MEN1* gene [28-31]. A loss of the 11p13 region has been described in some adenomas [32], and adenomas have been described in tuberous sclerosis [33].

10.4 Diagnostic Tests

The presence and severity of HI can be evaluated from the rate of glucose administration required to maintain normal glycaemia and the response to medical treatment. Both the severity of HI and its response to treatment depend upon the age at presentation [3].

10.4.1 Diagnostic Criteria

The diagnostic criteria for persistent HI include (1) fasting and postprandial hypoglycaemia (<3 mmol/l) persisting through the 1st month of life and associated with hyperinsulinaemia (plasma insulin >3mU/l), requiring high rates of i.v. glucose administration (>10 mg/kg/min) to maintain blood glucose >3 mmol/l; (2) an increase in blood glucose level of 2-3 mmol/l following s.c. or i.m. glucagon (0.5 mg). Nevertheless, in infancy and childhood, normal plasma insulin and even C-peptide concentrations during hypoglycaemia do not exclude the diagnosis of HI, and measurements must be repeated. In the absence of clearly abnormal insulin levels during a hypoglycaemic episode, an 8- to 12-h fasting test aimed at identifying an inappropriately small increase of plasma levels of ketone bodies, free fatty acid and branched-chain amino acids can be helpful [34].

Hyperammonaemia needs to be excluded in new patients with HI before a decision is reached to pursue a more aggressive treatment, since the HI/HA syndrome is usually amenable to medical or dietetic treatment. Similarly, analysis of urine organic acids and plasma acylcarnitines must be undertaken to investigate for fatty acid oxidation defects. In this context the finding of a 3-OH-butyryl-carnitine accumulation in plasma is highly suggestive of SCHAD deficiency. Finally, the secondary causes of HI should be excluded, namely FII, autoimmunity, and congenital disorders of glycosylation.

10.4.2 Differentiation of Focal from Diffuse Forms

Patients who are treated surgically should be classified according to histological criteria. The focal form, which accounts for 40% of the patients treated surgically, is defined as a focal adenomatous hyperplasia [35, 36]. The lesion measures 2.5-7.5 mm in diameter, differing from true adult-type pancreatic adenoma, which is more limited with a different topographic distribution. Diffuse HI shows abnormal β-cell nuclei in all sections of the whole pancreas [36]. In the absence of any distinctive clinical feature, and because preoperative classic radiology of the pancreas, including echotomography, CT scan and NMR, is not an efficient way of screening for the focal form, until recently pancreatic venous catheterisation (PVS) and pancreatic arteriography were the only preoperative procedures available for locating the site of insulin secretion [37].

However, the use of [^{18}F]-labelled fluoro-L-DOPA whole-body positron emission tomography (PET) has now been evaluated for the detection of hyperfunctional islet pancreatic tissue: an abnormal focal accumulation of [^{18}F]-labelled fluoro-L-DOPA is observed in the pancreas of the patients with a focal lesion, while a diffuse uptake of the radiotracer is observed over the whole pancreas in those with diffuse insulin secretion [38-41]. This new test, an accurate noninvasive technique, has now replaced PVS for the correct localisation of focal lesion in children.

[^{18}F]-Labelled fluoro-L-DOPA whole-body PET appears to be more reliable and easier to perform than the tolbutamide test that was proposed as a method for separating focal from diffuse forms of HI [42, 43].

10.5 Treatment and Prognosis

10.5.1 Medical Treatment

Treatment must be rapid and aggressive to prevent irreversible brain damage; this often necessitates central venous access and continuous oral alimentation using a nasogastric tube. Glucagon given continuously i.v. (1-2 mg per day) can be added if blood glucose levels remain unstable despite a high glucose infusion rate.

At the same time, specific treatments must also be given. Oral diazoxide should be used to treat HI, at a dose of 15 mg/kg/day in neonates and 10 mg/kg/day in infants, divided into three doses [3]. Diazoxide is usually effective in the infantile form (60% of cases in our experience), but most of those with the neonatal form are resistant to this treatment (90% of our cases).

Diazoxide efficacy is defined as the normalisation of blood glucose levels (>3 mmol/l) measured before and after each meal in patients fed normally with a physiological feed and after stopping i.v. glucose and any other medications for at least 5 consecutive days. Confirmed hypoglycaemia (<3 mmol/l) occurring twice in such a 24-h glucose cycle indicates a lack of response to diazoxide and requires continuous nasogastric drip feeding and/or other measures to be restarted. Tolerance to diazoxide is usually excellent. The most frequent adverse effect is hirsutism, which can sometimes be marked and distressing in young children. Haematological side effects and troublesome fluid retention are very rare with usual doses.

Octreotide can be tried before surgery in the case of unresponsiveness to diazoxide. Doses used have varied between 10 μg/day and 50 μg/day, given either in three or four s.c. injections or by s.c. pump [44]. High doses may lead to worsening of the hypoglycaemia by suppressing both glucagon and growth hormone. After starting treatment with octreotide, many patients have vomiting and/or diarrhoea and abdominal distension; however, these resolve spontaneously within 7-10 days. Steatorrhoea is also common; this partially responds to oral pancreatic enzymes and remits after several weeks to months. Gallbladder sludge can occur and necessitates routine abdominal ultrasound. Other drugs, such as calcium-channel blockers (e.g. nifedipine), have been proposed. All these treatments, if effective in controlling blood sugar, do not need to be increased according to the weight of the patient, so that their dose usually remains unchanged.

A restricted-protein diet, limiting the leucine intake to 200 mg per meal, is sometimes necessary in HI/HA syndrome, but diazoxide alone is often effective.

10.5.2 Surgical Treatment

Surgical treatment is required when medical or dietary therapies are ineffective or when a focal form is suspected. Previously, most paediatric surgeons recommended a 95% subtotal pancreatectomy in all such cases, a procedure which is associated with a high risk of subsequent development of diabetes mellitus [45]. There is now strong evidence that DiHI and FoHI require different surgical treatment [19, 46], even if the long-term mental prognosis is mostly related to the duration of the initial hypoglycaemia [42].

Intraoperative histology is performed to substantiate with certainty the findings of PET-CT and to guide the limits of resection in those confirmed to have FoHI. For these purposes, pancreatic samples must be collected

from the head, the isthmus, the body and the tail of the pancreas and immediately examined microscopically. DiHI lesions are characterised by ß-cells with large nuclei and abundant cytoplasm in all samples. The histological analysis of focal lesions shows no abnormal ß-cell nuclei, and shrunken cytoplasm giving a pattern of crowded ß-cells. In that case, additional samples are taken to localise the lesion, guided by the PET-CT. The localisation of focal forms is crucial in view of the fact that these can be located in the head of the pancreas, whereas surgeons usually resect pancreatic tissue by first removing its tail and body. After performance of a partial pancreatectomy a further series of samples is examined to ensure that the limits of resection are within normal pancreatic tissue. A subtotal pancreatectomy is performed for diffuse lesions. In addition to the two well-defined forms of HI, there are some patients who are not well characterised, having neither the typical focal nor the typical diffuse disease, but a mosaicism of their pancreatic cells [27]. In such forms it is usually prudent to restrict the size of the pancreatic resection.

10.5.3 Prognosis

Although most of the patients treated medically remain dependent on medication, some who respond well to medical management (diazoxide and/or octreotide) may undergo a complete clinical remission, relatively rapidly in the case of a focal lesion (<16 months) and later in the diffuse form. This justifies stopping medical treatment once a year under medical supervision to see whether there has been a spontaneous recovery. A conservative attitude is preferable for patients with HI/HA, who usually respond to diazoxide or a low-leucine diet and whose disorder is likely to recover spontaneously. However, neurological sequelae or epilepsy and mental retardation are possible [47], especially in HI/HA [6, 48].

Patients with FoHI treated by limited pancreatectomy are completely cured [21, 46]. In contrast, in those with DiHI postoperative hypoglycaemia and/or diabetes mellitus or serious alteration of glucose tolerance often follow subtotal pancreatectomy despite extensive surgery [47]. Pancreatic exocrine insufficiency may be treated by pancreatic enzyme replacement. An annual investigation of residual insulin secretion, based on pre- and postprandial plasma glucose and insulin levels at various intervals, as well as measurement of glycated haemoglobin (HbAIc) and an oral glucose tolerance test (OGTT), is mandatory, as diabetes or glucose intolerance can develop later. In the case of atypical lesions, a partial pancreatectomy can cure hypoglycaemia, or the patients remain hypoglycaemic.

10.6 Conclusion

In conclusion, the recommended strategy for investigation and management is as follows:

1. Exclude a transient form (<1 month) and secondary hyperinsulinism (FII, auto-immunity, CDG, overgrowth syndromes).
2. Assess for fasting and postprandial hyperammonaemia, an indication of HI/HA syndrome, and for plasma butyryl-carnitine, an indication for SCHAD.
3. Maintain blood glucose between 3 and 6 mmol/l with appropriate methods, including continuous drip feeding, i.v. glucose infusion, central line catheter, continuous i.v. glucagon.
4. Assess diazoxide and octreotide responsiveness, then dietary treatment.
5. Having excluded transient, secondary and familial forms and patients with hyperammonaemia and SCHAD, locate focal insulin secretion by [^{18}F]-labelled fluoro-L-DOPA PET.
6. Treat surgically those patients who are resistant to medical and dietary treatment and those in whom a focal form is strongly suspected.
7. Where surgery is required, verify the histological type of lesion by intraoperative histology, and perform a subtotal pancreatectomy for a diffuse lesion, or a limited pancreatectomy for a focal lesion.
8. Undertake molecular analysis (leukocyte and pancreatic DNA) in order to provide accurate genetic counselling and prenatal diagnosis.

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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 must have evolved: proteins embedded in the cell membrane function as hydrophilic pores that allow cellular uptake and release, and also transcellular 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.

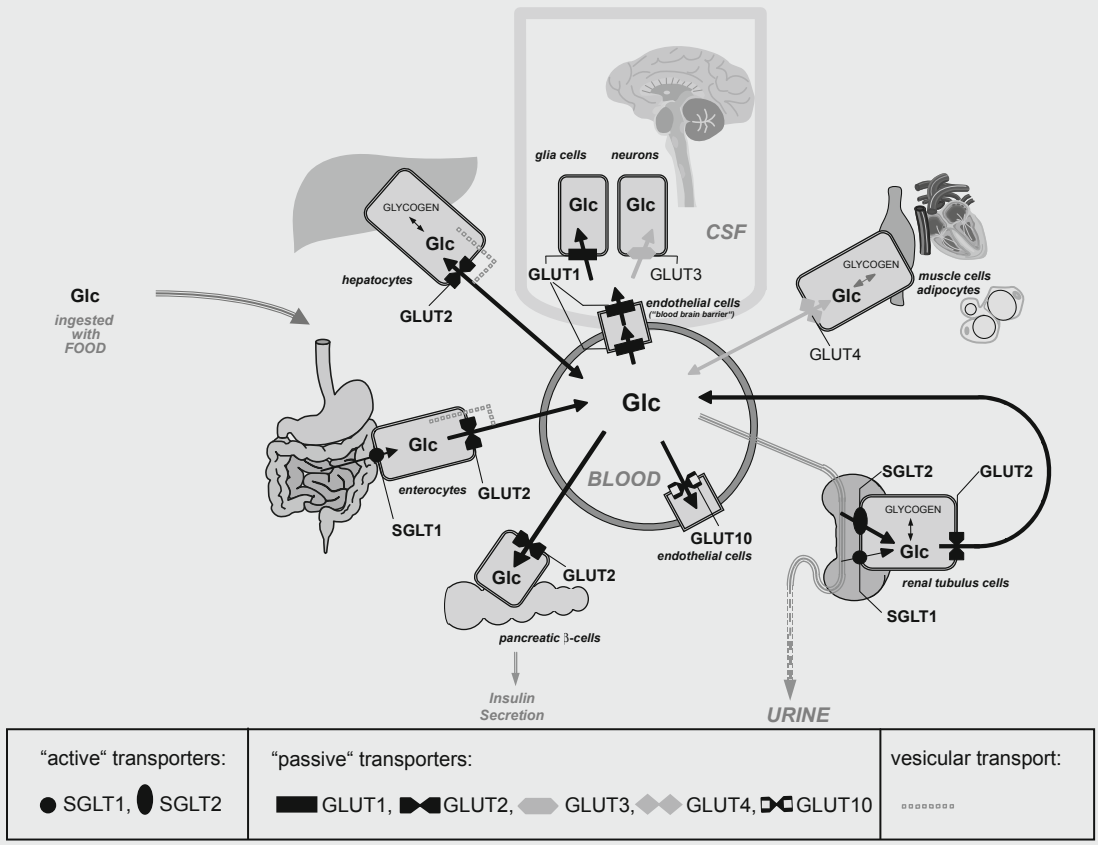

Fig. 11.1. Major routes of glucose transport. Transport across cell membranes is depicted by *arrows*, and specific transporters by *symbols*: *round* for sodium-dependent, 'active' transporters (SGLTs, encoded by members of the *SLC5* gene family), and *angular* for facilitative, 'passive' transporters (*GLUTs*, encoded by members of the *SLC2* gene family). Known defects are depicted in *black* instead of *grey transporter symbols* (▶ for details see text)

To date, five congenital defects of monosaccharide transporters are known (Fig. 11.1). Their clinical picture depends on tissue-specific expression and substrate specificity of the affected transporter. 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 deficiency syndrome, 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 within hepatic, renal and pancreatic 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 a novel entity characterised by hyperelastic connective tissue and generalised tortuosity and elongation of all major arteries including the aorta.

11.1 Congenital Glucose/Galactose Malabsorption (SGLT1 Deficiency)

11.1.1 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 following a normal pregnancy (with no polyhydramnion). Stools are very loose and may be mistaken for urine. Both breast- and bottle-fed infants are affected, but symptoms may already 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].

11.1.2 Metabolic Derangement

Congenital deficiency of SGLT1 is the basic defect in this disorder [4]. SGLT1 plays a complex role in the regulation of intestinal monosaccharide transport. It is a high-affinity, low-capacity sodium-dependent transporter in the brush border of enterocytes. Its presence, however, is also necessary to promote the postprandial expression of the facilitative transporter GLUT2 at the apical membrane. Thus, SGLT1 has a pivotal role in transepithelial transport of glucose and galactose, even though the bulk of these monosaccharides is absorbed by the facilitative component of transport [5]. At the basolateral membrane, glucose transport is mediated by facilitative diffusion and/or by a membrane vesicle-associated transport process [6]. 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 9).

Both truncating and missense mutations of the *SGLT1* (*SLC5A1*) gene 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 important physiological role of this transporter in renal glucose reabsorption.

11.1.3 Genetics

GGM is a relatively rare autosomal recessive disorder, although its exact prevalence is unknown. SGLT1, located on chromosome 22q13, codes for a protein of 664 amino acids that form 14 transmembranous loops [8]. To date, approximately 60 different mutations have been found [4, 7], scattered all over the gene; the existence of a mutational hotspot is controversial.

11.1.4 Diagnostic Tests

Owing to its life-threatening character, GGM must be suspected clinically and treatment started before the diagnosis can be confirmed. Clinical stabilisation with parenteral nutrition and no foods given orally or with 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 evoke 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 in a future pregnancy [9].

11.1.5 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 9).

11.2 Renal Glucosuria (SGLT2 Deficiency)

11.2.1 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 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.

11.2.2 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 counterregulatory hormones [12]); they can present with a delay of somatic maturation [13].

11.2.3 Genetics

Most individuals with renal glucosuria have been found to carry mutations within the *SGLT2* (*SLC5A2*) gene [14] located on chromosome 16p11 [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 [16]. Therefore, inheritance of renal glucosuria is best characterised by a codominant trait with variable expressivity. To date, approximately 50 *SGLT2* mutations have been described, which are scattered all over the gene [12, 16].

11.2.4 Diagnostic Tests

Diagnosis is straightforward in patients with glucosuria and normoglycaemia who do not show any other evidence of renal tubular dysfunction.

11.2.5 Treatment and Prognosis

In most cases dietary treatment is not indicated, and the prognosis, even in individuals with type 0 glucosuria, is excellent [13].

11.3 Glucose Transporter Deficiency Syndrome (GLUT1 Deficiency)

11.3.1 Clinical Presentation

GLUT1 deficiency syndrome (GLUT1DS) in its classic form presents as an early-onset epileptic encephalopathy. Following an uneventful fetal and neonatal period, epilepsy develops within the 1st year of life. Seizures are of various types and frequency, often refractory to anticonvulsants, and sometimes aggravated by fasting. Global developmental delay and a complex motor disorder (► below, »Nonclassic Phenotype«) become apparent, and in severe cases secondary microcephaly may develop [17, 18].

Nonclassic GLUT1DS presents as a complex movement disorder without epilepsy. Patients may develop an ataxic-spastic gait, action limb dystonia, mild chorea, and cerebellar action tremor or dystonic tremor. Additional features are myoclonus and dyspraxia. Nonepileptic paroxysmal events with episodes of ataxia, weakness, parkinsonism, alternating hemiplegia and nonkinesogenic dyskinesias may occur and may be triggered by poor dietary compliance and low ketonuria [18-20]. Manifestations with only minimal symptoms in adults have also been described [18, 21, 22].

Paroxysmal exertion-induced dystonia (PED) is allelic to GLUT1DS. Differences from GLUT1DS are an onset

beyond childhood, normal head circumference, exertion-induced dystonia with normal interictal neurological examination, and a less prominent decrease of the glucose concentration in cerebrospinal fluid (CSF) [23-25].

11.3.2 Metabolic Derangement

GLUT1 is a membrane-spanning, glycosylated protein that facilitates glucose transport across the blood-brain barrier. 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 [26, 27]. *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* [25].

11.3.3 Genetics

Approximately 80% of patients are heterozygous for mutations within the *GLUT1* (*SLC2A1)* gene located on chromosome 1p35-31.3. Autosomal dominant and autosomal recessive inheritance has been described [28-31]. Mutations are mostly de novo, of various character (missense, nonsense, and splice-site mutations, haploinsufficiency, compound heterozygosity and paternal mosaicism) and randomly distributed [17, 18, 31]. Emerging mutational hotspots have implications for *GLUT1* function. The type of mutation and the extent of hypoglycorrhachia is related to the phenotype; missense mutations and higher CSF glucose concentrations appear to be associated with a milder clinical presentation [18, 19].

11.3.4 Diagnostic Tests

GLUT1DS illustrates the importance of CSF evaluation in children with undiagnosed epilepsy and/or movement disorders. GLUT1DS 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 they appear to be higher in milder phenotypes and paroxysmal movement disorders [17-19]. A CSF-to-blood glucose ratio, which is normally >0.6, should be obtained in a nonictal, 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 and protein and lactate concentrations are normal [17-19].

Routine laboratory analyses are unremarkable, and interictal EEGs are often normal. If they are 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 [32]. 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, GLUT1DS 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 are generally reduced to about half control values [34] and do not correlate with disease severity.

11.3.5 Treatment and Prognosis

During fasting, ketone bodies provide an alternative fuel to the brain. This metabolic state is mimicked by a high-fat, low-carbohydrate ('ketogenic') diet that may restore brain energy metabolism in patients with GLUT1DS. Classic ketogenic diets (3:1 and 4:1 ratios of calories from fat and nonfat sources, respectively) and the modified Atkins diet may effectively control seizures and improve movement disorders and development [35, 36]. Multivitamin and calcium supplements are essential [37]. In contrast to intractable childhood epilepsy, in GLUT1DS the ketogenic diet 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 [38]. If an antiepileptic medication is required, carbamazepine, phenytoin or zonisamide should be considered. Valproate interferes with *GLUT1* function in vitro, but may be used in GLUT1DS [39, 40]. The use of dietary antioxidants, such as α-lipoic acid (thioctic acid), in GLUT1DS has been discussed. This drug, however, awaits further evaluation, and currently it cannot be considered an accepted therapy.

GLUT1DS has a favourable prognosis. Patients continue to make progress, acquiringe speech and mobility, and the disease generally stabilises after puberty. How-

ever, seizures and a variable degree of impairment may persist in some individuals despite adequate treatment [39], and a sheltered environment is often required.

11.4 Fanconi-Bickel Syndrome (GLUT2 Deficiency)

11.4.1 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 [41, 42]. A few cases have presented during neonatal screening owing to hypergalactosaemia [43], and cataracts have occasionally been observed as the first sign [44]. At an early stage, hepatomegaly, which is caused by massive accumulation of glycogen, may not yet be present, and nonspecific 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 more and more similar to that of patients with hepatic glycogen storage diseases. 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. FBS patients have an entirely normal mental development, but growth and puberty are severely retarded [41, 42].

11.4.2 Metabolic Derangement

Fanconi-Bickel syndrome is caused by congenital deficiency or 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. GLUT2 is further found at the apical and basolateral membrane of enterocytes and within the cell membrane of pancreatic β-cells.

Intestinal uptake of glucose and galactose appear unimpaired in FBS; this has been explained by an additional transport system for glucose, SGLT1 in the apical membrane and a membrane vesicle-associated pathway at the basolateral membrane [6]. Postprandial hyperglycaemia and hypergalactosaemia are caused by impaired hepatic uptake of the two sugars. There is increasing evidence that hyperglycaemia is further exaggerated by a diminished insulin response caused by an impairment of glucose sensing of β-cells [46]. In hepatocytes GLUT2 seems to function as a glucose sensor. Therefore, 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 [41].

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.

11.4.3 Genetics

FBS is a very rare autosomal recessive condition caused by mutations of the *GLUT2* (*SLC2A2*) gene. More than 70% of cases come from consanguineous families [47]. The human *GLUT2* gene, mapped to chromosome 3q26, 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 [48] and to date, more than 100 different mutations scattered throughout the gene have been detected [41, 47].

11.4.4 Diagnostic Tests

A 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 [49]. 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 histological 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 of 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 [41].

The diagnosis of FBS is ultimately confirmed by the detection of homozygosity or compound heterozygosity for *GLUT2* mutations [47].

11.4.5 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 [50].

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 [41].

11.5 Arterial Tortuosity Syndrome (GLUT10 Deficiency)

11.5.1 Clinical Presentation

This 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.

11.5.2 Metabolic Derangement

The role of a glucose transporter is as yet unknown in this condition, which more closely resembles a connective tissue disorder in presentation. Vascular problems are the consequence of the disruption of elastic fibres in the medial layer of the arterial wall. Deficiency of the facilitative glucose transporter GLUT10, which was detected by linkage analysis, 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 [51].

11.5.3 Genetics

Arterial tortuosity syndrome is a rare, recessively inherited condition. The gene encoding the affected transporter, *GLUT10* (*SLC2A10*), is a member of the so-called type III *GLUT*s, which are older and more distantly related to the previously mentioned facilitative glucose transporter genes. *GLUT10* has been located to 20q13.1, and to date approximately 20 different mutations have been described [51, 52].

- **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.

11.5.4 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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III Disorders of Mitochondrial Energy Metabolism

Disorders of Pyruvate Metabolism and the Tricarboxylic Acid Cycle

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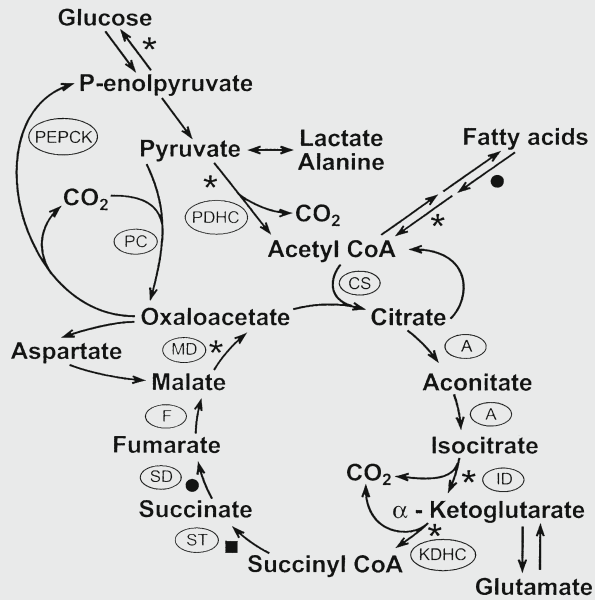
Pyruvate Metabolism and the Tricarboxylic Acid Cycle

Pyruvate is formed from glucose and other monosaccharides, from lactate, and from the gluconeogenic amino acid alanine (◘ Fig. 12.1). After entering the mitochondrion, pyruvate can be converted into acetylcoenzyme 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 and reduced flavin adenine dinucleotide, which are utilised to produce energy under the form of ATP in the electron transport chain.



◘ **Fig. 12.1.** 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 following symbols: *, reduced nicotinamide adenine dinucleotide; ●, reduced flavin adenine dinucleotide; ■, guanosine triphosphate

Owing to the role of pyruvate and the tricarboxylic acid (TCA) cycle in energy metabolism, as well as in gluconeogenesis, lipogenesis and amino acid synthesis, defects in pyruvate metabolism and in the 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 15). 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 phosphoenolpyruvate carboxykinase (PEPCK) is now considered to be a secondary phenomenon. Deficiency of the 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) or minimising dietary carbohydrate intake (PDHC) 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 19), with biochemical 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.

12.1 Pyruvate Carboxylase Deficiency

12.1.1 Clinical Presentation

Three phenotypes are associated with pyruvate carboxylase deficiency.

The patients with French phenotype (type B) become acutely ill 3-48 h after birth with hypothermia, hypotonia, lethargy and vomiting [1-6]. 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 Parkinson disease) and abnormal ocular movements [5]. 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.

The patients with North American phenotype (type A) become severely ill between 2 and 5 months of age [2, 6, 7]. 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 constant finding. Clinical examination reveals pyramidal tract signs, ataxia and nystagmus. All patients are severely mentally retarded, and most of them have convulsions. Hepatomegaly and renal dysfunction might 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 [4]. The course of the disease is generally downhill, with death in infancy.

A third form, more benign, is rare and has only been reported in a few patients [8, 9]. 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 near-normal cognitive and neuromotor development. However subcortical leukodystrophy has been described in some cases.

12.1.2 Metabolic Derangement

PC is a biotinylated mitochondrial matrix enzyme that converts pyruvate and CO_2 to oxaloacetate (◘ Fig. 12.1). 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 phosphoenol-pyruvate by phosphoenol-pyruvate carboxykinase (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 [6]. 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 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 [4].

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 paraventricular cavities around the frontal and temporal horns of the lateral ventricles, reported in the few detailed neuropathological descriptions of PC deficiency [1, 4]. There is an astroglia-like location of PC [10]. PC is present in oligodendrocytes (glial cells essential for the formation of myelin sheets), where it plays an anaplerotic role [11]. 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 [12].

PC requires biotin as a cofactor. Metabolic derangements of PC deficiency are thus also observed in biotin-responsive multiple carboxylase deficiency (► Chapter 27).

12.1.3 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 (B) have absence of PC protein and of the corresponding mRNA. The patients with North American phenotype (A) generally have cross-reacting material (CRM-positive) [2], as did the patient with the benign variant of PC deficiency [8]. The PC gene contains 19 exons and 18 introns [7].

Mutations have been detected in patients of both types A and B. In Canadian Indian populations with type A disease, 13 patients were homozygous for a 1828G→A missense mutation (Ala610Thr) in exon 13 of the *PC* gene [13]. Several reports on mutations are now published [13-16].

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.

12.1.4 Diagnostic Tests

The possibility of PC deficiency should be considered in any child presenting with lactic acidosis and neurological abnormalities, especially if associated with hypoglycaemia, hyperammonaemia or ketosis. In neonates, a high lactate/pyruvate ratio associated with a low 3-OH-butyrate/acetoacetate ratio and hypercitrullianemia with low glutamate/glutamine is nearly pathognomonic [5]. 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 the French phenotype, blood lactate concentrations reach 10-20 mM (normal <2.2 mM) with lactate/pyruvate ratios between 50 and 100 (normal <28). In patients with the North American phenotype, blood lactate is 2-10 mM with normal or only moderately increased lactate/pyruvate ratios (<50). In patients with the benign type, lactate can be normal and only increase (usually above 10 mM) during acute episodes. Overnight blood glucose concentrations are usually normal, but decrease after a 24-h fast. Hypoglycaemia can occur during acute episodes of metabolic acidosis. Blood 3-OH-butyrate is increased (0.5-2.7 mM, normal <0.1) and 3-OH-butyrate/acetoacetate ratio is decreased (<2, normal 2.5-3).

Hyperammonaemia (100-600 μM, normal <60) and an increase of blood citrulline (100-400 μM, normal <40), lysine and proline, contrasting with low glutamine, are constant findings in patients with the French phenotype [5]. Plasma alanine is usually normal in the French phenotype, but increased (0.5-1.4 mM, normal <0.455) in all reported patients with the North American phenotype. During acute episodes, aspartate can be undetectably low [17].

In cerebrospinal fluid (CSF), lactate, the lactate/pyruvate ratio and alanine are increased and glutamine is decreased.

Measurement of the activity of PC is preferentially performed on cultured skin fibroblasts [6]. 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. PC has low activity in skeletal muscle, which means this tissue is not useful for assay. PC activity in fibroblasts is severely decreased, to less than 5% of normal, in all patients with the French phenotype, varies from 5% to 23% of controls in patients with the North American phenotype, and is less than 10% of controls in patients with the benign variant. Although these three types are described, it is possible that they form part of a continuum.

Prenatal diagnosis of PC deficiency is possible by measurement of PC activity in cultured amniotic fluid cells [18], direct measurement in chorionic villi biopsy specimens [3] or DNA analysis when the familial mutations are known.

12.1.5 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 [17] lactate and ketones diminished and plasma amino acids normalised, 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 [19]. A patient with the French phenotype was started on early treatment with triheptanoin (4 g of triheptanoin/kg body weight, providing 35% of total caloric intake) in order to restore anaplerosis [20].

Lactate, the lactate/pyruvate ratio, ammonia, and citrulline decreased rapidly, and there was 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 8 months of age. Neither biotin, thiamine, dichloroacetate, nor a high-fat or high-carbohydrate diet has been shown to provide clinical benefit.

The prognosis of patients with PC deficiency depends on the severity of the defect. Patients with minimal re-

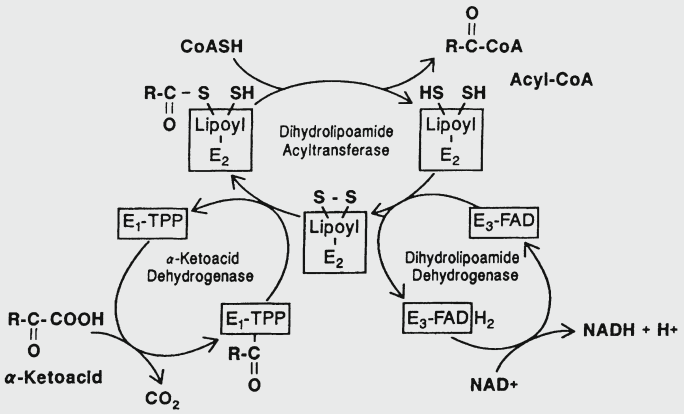
sidual PC activity usually do not live beyond the neonatal period, but some children with very low PC activity have survived to more than 5 years. Those with milder defects or somatic mosaicism [15] may survive longer with varying degrees of neurological disease.

12.2 Phosphoenolpyruvate Carboxykinase Deficiency

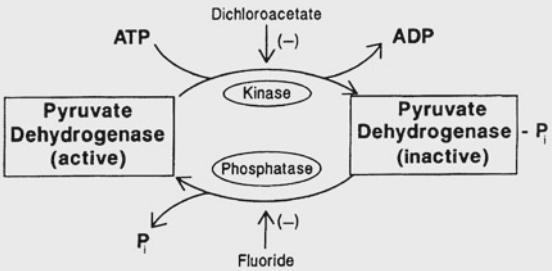
Phosphoenolpyruvate carboxykinase (PEPCK) deficiency was first described by Fiser et al. [21]. Since then, only five additional patients have been reported in the literature, and none since 1986 [22]. It now appears that deficiency of PEPCK is more likely to be a secondary phenomenon. Separate mitochondrial and cytosolic isoforms of the enzyme exist. The cDNA encoding the cytosolic isoform of PEPCK in humans has been sequenced and localised to chromosome 20; 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 [23]. Deficiency of mitochondrial PEPCK has also been disputed: a sibling of a patient developed a similar clinical picture but had normal enzyme activity [23]. Further studies showed a depletion of mitochondrial DNA in this patient [24] caused by defective DNA replication [25].

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 E3BP, E3-binding protein, formerly protein X. This component has its role in attaching E3 subunits to the core of E2 (◘ Figs. 12.2, 12.3).



◘ **Fig. 12.2.** 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. 12.3.** Activation/deactivation of PDHE1 by dephosphorylation/phosphorylation. Dichloroacetate is an inhibitor of E1 kinase, and fluoride inhibits E1 phosphatase. *ADP,* adenosine diphosphate; *P,* phosphate

12.3 Pyruvate Dehydrogenase Complex Deficiency

12.3.1 Clinical Presentation

More than 200 cases of pyruvate dehydrogenase complex (PDHC) deficiency have been reported [26-29], the majority of which involves the X-encoded α subunit of the dehydrogenase component (E1) of the complex (◘ Fig. 12.2). 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 is initially much less severely affected, with intermittent episodic ataxia after carbohydrate-rich meals, progressing slowly over years into mild Leigh's encephalopathy. A number of patients have developed an acute peripheral neuropathy during infancy [30], or an acute episodic ataxia [31], without mental retardation. Optic atrophy can also be a late finding (► Chapter 2).

Females with PDHE1α deficiency tend to have a more uniform clinical presentation, although with variable severity, depending on variable lyonisation [32]. This includes dysmorphic features, microcephaly, moderate to severe mental retardation and spastic di- or quadriplegia, resembling nonprogressive 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 and partial to complete corpus callosum agenesis [33]. Severe neonatal lactic acidosis can be present. The difference in the presentation of PDHE1α deficiency in boys and girls is exemplified by observations in a brother and sister pair with the same mutation but completely different clinical features [32]. Males [34-36] and females [37] who are mosaic for PDHE1α deficiency have been reported with an attenuated phenotype.

Neuroradiological abnormalities such as corpus callosum agenesis and dilated ventricles, or in boys basal ganglia and midbrain abnormalities, are often found. Neuropathology can reveal various degrees of dysgenesis of the corpus callosum. This is 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 [38].

Only a few cases with PDHE1β deficiency have been reported [39-42]. These patients present with early-onset lactic acidosis and severe developmental delay. A moderate clinical course with slowly progressive neurological features reflecting basal ganglia and brain stem involvement associated with typical findings of Leigh syndrome has also been reported [42]. Seven cases of E1-phosphatase deficiency [43] (◘ Fig. 12.3) have been identified, including two brothers with hypotonia, feeding difficulties and delayed psychomotor development [44]. A lethal infantile phenotype has recently been described [45]. This novel mechanism of PDHC deficiency may not have been fully investigated before [46]. A few cases of PDHE2 (dihydrolipoamide transacetylase) deficiency have been reported recently [47, 48]. The main clinical manifestations of E3BP (formerly protein X) deficiency are hypotonia, delayed psychomotor development and prolonged survival [49-51]. Often more slowly progressive, it also comprises early-onset neonatal lactic acidosis associated with subependymal cysts and thin corpus callosum.

12.3.2 Metabolic Derangement

Defects of PDHC provoke conversion of pyruvate into lactate and alanine rather than in acetyl-CoA, the gateway for complete oxidation of carbohydrate via the TCA cycle (◘ Fig. 12.1). 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 reduced nicotinamide adenine dinucleotide (NADH) but, unlike respi-

ratory 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 because of impaired NADH oxidation.

12.3.3 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 Xp22.12. Therefore, most cases of PDHC deficiency are X-linked. To date, over 120 different mutations of the E1α subunit of PDHC have been characterised [52-54]. 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 [52]. No null E1α mutations have been identified in males, except in a mosaic state [34], suggesting that such mutations are likely to be lethal. Rare splicing mutations involve exonic [55-57] or intronic [58] regulatory sequences. A few large rearrangements have been identified, such as a large intragenic 5 kb deletion [59].

Only nine E1β-deficient patients have been described, with mutations in the *PDHB* gene in eight of them [39-42]. The deficiency in the ninth patient was due to an increased proteasome-mediated degradation of the ubiquinated E1β subunit [40]. The molecular basis of E3-binding protein (E3BP) deficiency has been characterised in 20 cases. Half of these patients have splicing errors, while others have frameshift or nonsense mutations [60, 61]. Two large rearrangements have been identified, one of them due to retrotranspositional insertion of a full-length LINE-1 element [62]. Recently mutations in E2 [47, 48] and in the pyruvate dehydrogenase phosphatase gene (*PDP1*) [44-46] have been identified.

In only about 25% of cases was the mother of a child with PDHE1α deficiency a carrier of the mutation [52]. 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.

12.3.4 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 [63]. 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). Glucose tolerance or carbohydrate loading tests are not necessary for a definite diagnosis. In contrast to deficiencies of PC or PEPCK, fasting hypoglycaemia is not an expected feature of PDHC deficiency, and blood lactate and pyruvate usually decrease after fasting [63].

The most commonly used material for assay of PDHC is cultured skin fibroblasts [64]. PDHC can also be assayed in lymphocytes, separated from EDTA-blood, after less than 2 days. Molecular analysis of the *PDHE1*α gene in girls is often more efficient than measuring the enzyme activity. If available, skeletal muscle and/or other tissues are useful [65, 66]. PDHC is commonly assayed by measuring the release of $^{14}CO_2$ from [1-^{14}C]pyruvate in cell homogenates and tissues [67]. PDHC activity should be measured at low and high TPP concentrations to detect thiamine-responsive PDHC deficiency [68].

PDHC must also be activated (dephosphorylated; ◘ Fig. 12.3) 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. 12.3). In E1-phosphatase deficiency there is a deficiency in native PDH activity, but on activation of the PDH complex with DCA, activity becomes normal [43]. The three catalytic components of PDHC can be assayed separately. Immunoblotting of the components of PDHC can help distin-

guish whether a particular protein is missing. In females with PDHE1α deficiency, X inactivation can interfere with the biochemical analysis [63]. E3BP, which anchors E3 to the E2 core of the complex, can only be evaluated using immunoblotting, since it has no catalytic activity [49].

12.3.5 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 [69]. 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 [70]. Thiamine has been given in variable doses (500-2,000 mg/day), with lowering of blood lactate and apparent clinical improvement in some patients [71, 72].

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. 12.3). 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 [73]. 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 [74]. 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.

12.4 Dihydrolipoamide Dehydrogenase Deficiency

12.4.1 Clinical Presentation

Approximately 25 cases of E3 deficiency have been reported [75-78]. Since this enzyme is common to all the 2-ketoacid dehydrogenases (◘ Fig. 12.2), 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 [79].

12.4.2 Metabolic Derangement

Dihydrolipoyl dehydrogenase (E3) is a flavoprotein common to all three mitochondrial α-ketoacid dehydrogenase complexes (PDHC, KDHC and BCKD; ◘ Fig. 12.3). 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, and valine), and increased urinary lactic, pyruvic, 2-ketoglutaric, and branched-chain 2-hydroxy- and 2-ketoacids.

12.4.3 Genetics

The gene for E3 is located on chromosome 7q31-q32 [76], and the deficiency is inherited as an autosomal recessive trait. Mutation analysis in 13 unrelated patients has revealed 14 different mutations [76-79]. A G194C mutation is the major cause of E3 deficiency in Ashkenazi Jewish patients [80].

12.4.4 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 [81]. In cultured skin fibroblasts, blood lymphocytes, or other tissues, the E3 component can be assayed using a spectrophotometric method.

12.4.5 Treatment and Prognosis

There is no dietary treatment for E3 deficiency, since the affected enzymes effect carbohydrate, fat, and protein metabolism. Restriction of dietary branched-chain amino ac-

ids was reportedly helpful in one case [82]. DL-Lipoic acid has been tried, but its efficacy remains controversial [80].

12.5 2-Ketoglutarate Dehydrogenase Complex Deficiency

12.5.1 Clinical Presentation

Isolated deficiency of the 2-ketoglutarate dehydrogenase complex (KDHC) has been reported in several unrelated families [83, 84]. 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 [85]. All patients presented in the neonatal period and early childhood.

In one patient the clinical picture was milder [84]. 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 [86].

12.5.2 Metabolic Derangement

KDHC is a 2-ketoacid dehydrogenase that is analogous to PDHC and BCKD (Fig. 12.2). 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.

12.5.3 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.

12.5.4 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.

12.5.5 Treatment and Prognosis

There is no known effective treatment.

12.6 Fumarase Deficiency

12.6.1 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 [87].

Most of the patients present in infancy with a severe encephalopathy and seizures, with poor neurological outcome and profound mental retardation [88]. 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 nonprogressive 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 [88].

12.6.2 Metabolic Derangement

Fumarase catalyses the reversible interconversion of fumarate and malate (Fig. 12.1). 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. 12.1).

12.6.3 Genetics

Fumarase deficiency is inherited as an autosomal recessive trait. A single gene, mapped to chromosome 1q42.1, and the same mRNA, encode alternately translated transcripts to generate a mitochondrial and a cytosolic isoform [89]. A variety of mutations have been identified in several unrelated families [89-92]. Prenatal diagnosis is possible by enzyme assay and/or mutational analysis in CVS or cultured amniocytes [89]. Heterozygous mutations in the fumarase gene are associated with a predisposition to cutaneous and uterine leiomyomas and to kidney cancers [93].

12.6.4 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 [87].

12.6.5 Treatment and Prognosis

There is no specific treatment.

12.7 Succinate Dehydrogenase Deficiency

12.7.1 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. The clinical picture of this very rare disorder [94-97] 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 [98].

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 a paradoxically decreased L/P ratio during exercise. It can start with early-childhood-onset fatigue; episodic weakness and rhabdomyolysis may occur [99, 100].

12.7.2 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. 12.1) 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 [98]. 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 [97].

12.7.3 Genetics

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) have been mapped to chromosomes 5p15 and 1p35-p36, respectively, while the two integral membrane proteins (C and D) have been mapped to chromosomes 1q21 and 11q23. Homozygous and compound heterozygous mutations of SDA have been identified in several patients [95, 98-99], as well as mutations in a separate iron-sulfur cluster encoding gene [100]. 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 [101].

Mutations in SDB, SDC or SDD cause susceptibility to familial phaeochromocytoma and familial paraganglioma [102]. This suggests that *SD* genes may act as tumour suppression genes.

12.7.4 Diagnostic Tests

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 [98].

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. Magnetic resonance spectroscopy provides a characteristic pattern with accumulation of succinate [103].

12.7.5 Treatment and Prognosis

No effective treatment has been reported. Although SD is a flavoprotein, riboflavin-responsive defects have not been described.

12.8 Other Krebs Cycle Disorders

Recently, deficiency of mitochondrial NAD^+-specific isocitrate dehydrogenase (IDH3) was found to be associated with retinitis pigmentosa [104]. 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 23).

12.9 Pyruvate Transporter Defect

12.9.1 Clinical Presentation

Only one patient has been completely documented [105]. 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, as reported for the first patient (M. Brivet, personal data).

12.9.2 Metabolic Derangement

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.

12.9.3 Diagnostic Tests

As in PDHC deficiency, high lactate and pyruvate are found with normal lactate/pyruvate 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.

12.9.4 Genetics

Chromosome localisation and cDNA sequence of the pyruvate carrier is still unknown. Prenatal diagnosis on CVS can be done by the biochemical method [105].

12.9.5 Treatment and Prognosis

No treatment is known at this moment.

12.10 Protein-bound lipoid acid defect

A novel fatal mitochondrial disease associated with defective NFU1 function in the maturation of subset of mitochondrial Fe-S proteins have been recently described. All ten patients described so far developed normally in the early neonatal period and exhibited hypotonia, refusal to feed irritability and pulmonary hypertension. Hyperglycinemia, lactic acidosis and high excretion of 2-Ketoglutaric, 2-Ketoadipic, 2-Hydroadipic and glutaric acids were common findings. Diagnosis relies on molecular investigation of NFU_1 which encodes a protein involved in lipoylation of mitochondrial proteins [106].

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Disorders of Mitochondrial Fatty Acid Oxidation and Related Metabolic Pathways

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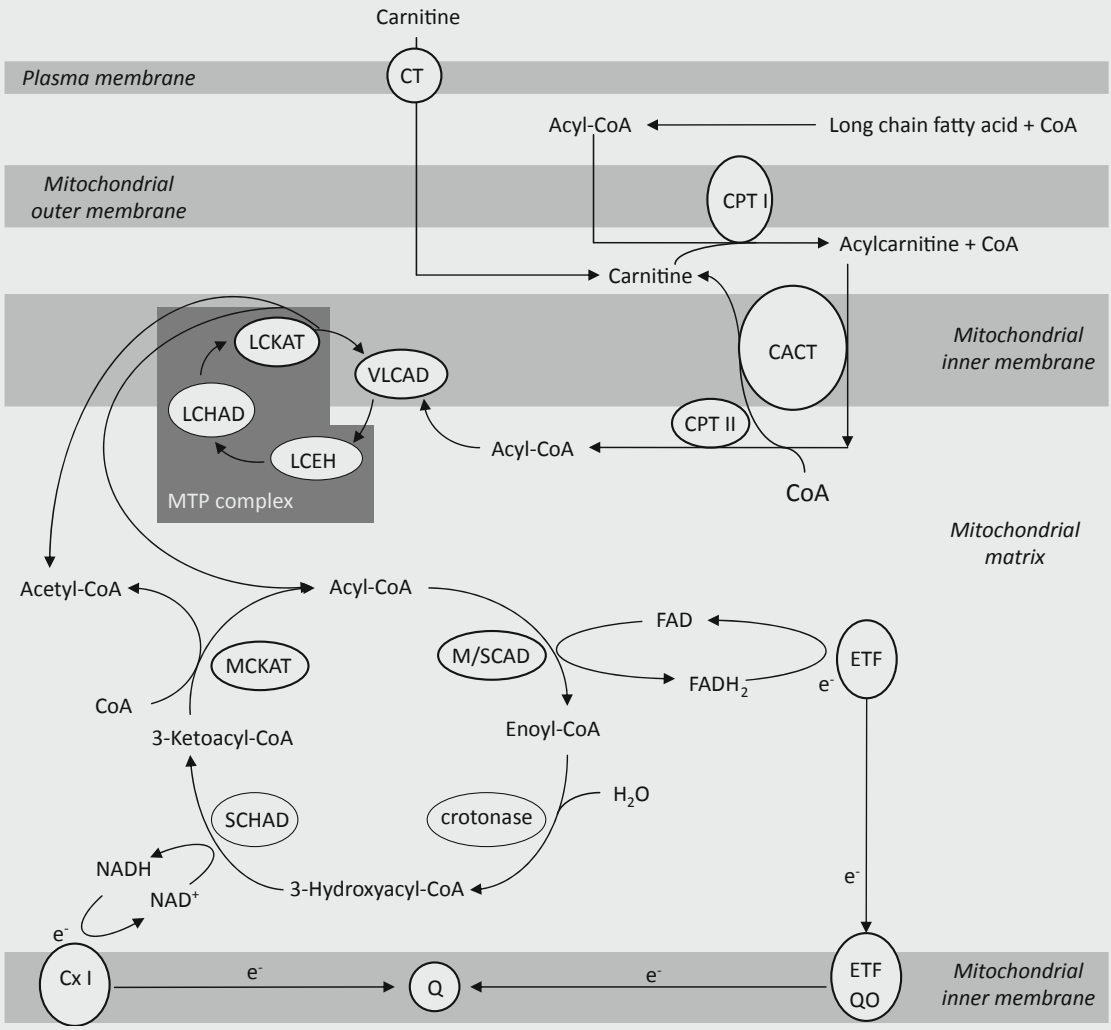
Mitochondrial Fatty Acid Oxidation

Mitochondrial fatty acid oxidation involves three processes (◘ Fig. 13.1):

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 esters within the mitochondria. Carnitine palmitoyltransferase I is the main site for the regulation of fatty acid oxidation. Medium and short-chain fatty acids enter mitochondria independently of carnitine and are activated to CoA esters in the matrix.
2. *ß-Oxidation via a spiral pathway*. Each turn of the spiral shortens the acyl-CoA by two carbons and in-



◘ **Fig. 13.1.** 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; *LCEH*, long-chain enoyl-CoA hydratase; *LCHAD*, long-chain 3-hydroxyacyl-CoA dehydrogenase; *LCKAT*, long-chain ketoacyl-CoA thiolase; *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; *SCHAD*, short-chain 3-hydroxyacyl-CoA dehydrogenase; *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). Defects have been identified in all the enzymes except crotonase and dodecenoyl-CoA delta isomerase

volves four steps. These include two dehydrogenation reactions, linked respectively to flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NAD). ß-Oxidation is catalysed by enzymes of different chain length specificities. The enzymes for long-chain substrates are membrane-bound and 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 $FADH_2$ to ubiquinone). Acetyl-CoA released by ß-oxidation can either be oxidised in the Krebs cycle or, in the liver, used to synthesise ketone bodies (▶ Chapter 14).

Fatty acid oxidation disorders have a high incidence in populations of European origin, though they are rarer in Asia. Many countries now have newborn screening programmes 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 leukotriene metabolism and Sjögren-Larsson syndrome.

13.1 Introduction

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 they 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.

13.2 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 [1].

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 13.1), depending on the residual enzyme activity and the age of the patient. Thus, patients may present with hypoglycaemia in infancy but only suffer 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; these patients have mainly been identified since the introduction of newborn screening and it is not yet clear how many will remain asymptomatic throughout life.

13.2.1 Fatty Acid Transport Defects

The mechanisms of fatty acid transport across the plasma membrane are still not completely clear [2]. Impaired uptake has been reported in two boys who presented with recurrent/fulminant liver failure, associated with hypoglycaemia and hyperammonaemia, but the underlying defect remains uncertain [3].

13.2.2 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 systemic carnitine deficiency with increased renal loss of carnitine, low plasma carnitine concentrations and intracellular concentrations that can be low enough to impair fatty acid oxidation.

Table 13.1. Inherited disorders of mitochondrial fatty acid oxidation

Defect	Potential clinical manifestations*				
	Hypoglycaemia and acute hepatic dysfunction	Cardiomyopathy	Acute rhabdomyolysis	Chronic weakness	Other problems
CT	+	+		+	
CPT I	+	+	+	+	RTA
CACT	+	+		+	Malformations
CPT II	+				
VLCAD	+	+	+	+	
MCAD	+	+	+	+	Retinopathy, Neuropathy
LCHAD/MTP	+				Hyperinsulinism
SCHAD	+				
MAD	+	+		+	Malformations

CACT, carnitine/acylcarnitine translocase; *CPT*, carnitine palmitoyltransferase; *CT*, carnitine transporter; *LCHAD*, long-chain 3-hydroxyacyl-CoA dehydrogenase, *MAD,* multiple acyl-CoA dehydrogenase; *MCAD*, medium-chain acyl-CoA dehydrogenase; *MTP,* mitochondrial trifunctional protein; *RTA,* renal tubular acidosis*; SCHAD*, short-chain 3-hydroxyacyl-CoA dehydrogenase; *VLCAD*, very-long-chain acyl-CoA dehydrogenase
*Individual patients may show all, some or none of these problems, depending on the residual enzyme activity and exposure to environmental stress

The commonest presentation is with heart failure, usually between 1 and 7 years of age [4]. Echocardiography shows thickened ventricular walls and reduced contractility. Without carnitine treatment, patients may die of rapidly deteriorating cardiac function. The cardiomyopathy is often accompanied by skeletal muscle weakness. Other patients present with hypoglycaemia or sudden death, precipitated by fasting or an infection, usually before the age of 2 years.

Neonatal screening has revealed a higher frequency of OCTN2 defects than was detected clinically, including some affected mothers, most of whom have been asymptomatic [5]. Clearly a number of patients remain healthy into adulthood despite very low plasma carnitine concentrations.

▪ 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 [6, 7]. Paradoxically, several patients have had cardiac problems or raised plasma creatine kinase (CK) [6].

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 [8].

▪ Carnitine-acylcarnitine Translocase (CACT) Deficiency

Approximately 30 patients with this disorder have been reported. Most have presented in the neonatal period and died by 3 months of age. Problems have included coma due to hypoglycaemia and hyperammonaemia, cardiomyopathy, atrioventricular block and ventricular arrhythmias [9, 10]. A few more mildly affected patients present with hypoglycaemic encephalopathy, precipitated by fasting or infections [11].

▪ Carnitine Palmitoyltransferase II (CPT II) Deficiency

The commonest form of this disorder is a mild deficiency that presents with recurrent episodes of rhabdomyolysis [12]. 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 also be brought on by infections. Muscle pain may start 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, associated with chronic weakness.

Severe neonatal onset CPT II deficiency is 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 [13]. There is also an intermediate form of

CPT II deficiency that causes episodes of hypoglycaemia and liver dysfunction, sometimes accompanied by cardiomyopathy and arrhythmias [14].

13.2.3 ß-Oxidation Defects

- **Very-long-chain Acyl-CoA Dehydrogenase (VLCAD) Deficiency**

This disorder has a wide clinical spectrum [15]. Mildly affected patients present as adolescents or adults with exercise-induced rhabdomyolysis [16]. Other patients present in childhood with episodes of hypoglycaemic encephalopathy; these patients may also suffer exercise- or illness-induced rhabdomyolysis or chronic weakness, usually at a later age. Severely affected patients present in early infancy with cardiomyopathy, in addition to the problems seen in milder patients [17]. 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 [18]. This is much higher than was detected clinically. Undoubtedly, the diagnosis was previously missed in some symptomatic patients, particularly those presenting as adults with rhabdomyolysis. It is likely, however, that many patients diagnosed by screening would remain asymptomatic without intervention (► Chapter 3).

- **Long-chain 3-Hydroxyacyl-CoA Dehydrogenase (LCHAD) and Mitochondrial Trifunctional Protein (MTP) Deficiencies**

MTP is composed of four α-subunits and four ß-subunits; the α-subunit has long-chain enoyl-CoA hydratase (LCEH) and LCHAD activities, and the ß-subunit has long-chain ketoacyl-CoA thiolase (LCKAT) activity. Patients can have isolated LCHAD deficiency or a generalised deficiency of all three enzyme activities.

Patients with isolated LCHAD deficiency usually present acutely with hypoglycaemia, most commonly between 1 and 6 months of age [19]. This is accompanied by liver dysfunction, lactic acidosis and, often, cardiomyopathy. Other patients present with chronic symptoms, such as failure to thrive, hypotonia or cardiomyopathy. A few patients have cholestasis or cirrhosis, which is unusual in fat oxidation disorders. Over subsequent years, there may be feeding problems, recurrent metabolic derangement, episodes of rhabdomyolysis or, occasionally, hypoparathyroidism [20]. Most patients develop retinopathy during childhood. Granular pigmentation, especially in the macular region, is followed by chorioretinal atrophy with deteriorating central vision and dark adaptation [21]. Some patients develop cataracts [21]. Peripheral neuropathy is a rarer long-term complication.

Generalised MTP deficiency is more heterogeneous [22]. Patients with severe deficiency present as neonates with hypoglycaemia, liver dysfunction and cardiomyopathy and 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 a peripheral neuropathy, which can present at any age from infancy to adulthood [23].

Mothers who are heterozygous for LCHAD or MTP deficiency have a high risk of illness during pregnancies when they are carrying an affected fetus [24]. The main problems are HELLP syndrome (*h*aemolysis, *e*levated *l*iver enzymes and *l*ow *p*latelets) and acute fatty liver of pregnancy (AFLP). HELLP syndrome has also been reported in some other FAODs but it is not clear whether the risk is genuinely increased.

- **Medium-chain Acyl-CoA Dehydrogenase (MCAD) Deficiency**

MCAD deficiency is much the commonest fatty acid oxidation disorder in Northwest Europe, with an incidence of approximately 1:12,000-20,000 in the UK, USA and Australia [25-27].

Patients usually present between the ages of 4 months and 4 years with acute hypoglycaemic encephalopathy and liver dysfunction. This is precipitated by prolonged fasting or, more often, by an infection with vomiting. Some patients present with sudden unexpected death, but a careful history generally reveals a preceding illness with poor feeding and lethargy. Affected babies, particularly if breast-fed, may present in a similar way within 72 h of birth [28]. Patients with MCAD deficiency only present clinically if exposed to an appropriate environmental stress. Even before newborn screening, many patients, probably 30-50%, had remained asymptomatic [25, 27]; with newborn screening and preventative measures, hypoglycaemia is rare. Patients do not develop cardiomyopathy or myopathy, and few present as adults [29].

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 h from 4-6 months of age prior to diagnosis. Encephalopathy can occur without hypoglycaemia [29], presumably due to the accumulation of free fatty acids and their carnitine and CoA esters.

- **Medium-chain 3-Ketoacyl-CoA Thiolase (MCKAT) Deficiency**

This defect has only been reported in one patient [30]. He presented at 2 days of age and died at 13 days, having developed hypoglycaemia, hyperammonaemia, acidosis and myoglobinuria.

- **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 [31]. 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. This suggests that SCAD deficiency should not be included in neonatal screening programmes.

- **Short-chain 3-Hydroxyacyl-CoA Dehydrogenase (SCHAD) Deficiency**

SCHAD deficiency is associated with hypoglycaemia due to hyperinsulinism, in contrast to other FAODs. This has been documented in five families, who presented in early infancy and responded to treatment with diazoxide [32] (► Chapter 10). There was low SCHAD activity in fibroblasts, and mutations were found in the *SCHAD* gene. The pathophysiology is independent of ß-oxidation. SCHAD has a second role, that of binding and inhibiting glutamate dehydrogenase (GDH): SCHAD mutations that prevent GDH binding lead to increased GDH activity and insulin secretion, particularly in response to leucine [33]. There is also a report of one SCHAD-deficient patient who presented at 10 months with a Reye-like illness but no hypoglycaemia; this patient had missense mutations with >35% residual activity in fibroblasts [34].

- **Acyl-CoA Dehydrogenase 9 (ACAD9) Deficiency**

ACAD9 is homologous to VLCAD and has dehydrogenase activity towards long-chain acyl-CoA esters in vitro. Its physiological role, however, consists in the assembly of respiratory chain complex I [35]. ACAD9 defects have been identified in five pedigrees with unexplained complex I deficiency; all the patients presented with hypertrophic cardiomyopathy and lactic acidaemia [35, 36].

- **Long-chain Acyl-CoA Dehydrogenase (LCAD) Deficiency**

Originally, patients with VLCAD deficiency were misdiagnosed as having long-chain acyl-CoA dehydrogenase (LCAD) deficiency. LCAD deficiency has been recently found in a premature baby presenting with a congenital surfactant deficiency (36 b).

- **2,4-Dienoyl-CoA Reductase Deficiency**

This defect of unsaturated fatty acid oxidation was suspected in a girl who had an abnormal unsaturated acylcarnitine in blood and urine. She presented with hypotonia and died of respiratory failure aged 4 months [37].

13.2.4 Electron Transfer Defects

- **Multiple acyl-CoA Dehydrogenase (MAD) Deficiency**

This disorder is also known as glutaric aciduria type II and results from defects of electron transfer flavoprotein (ETF) or ETF ubiquinone oxidoreductase (ETFQO). ETF and ETFQO carry electrons to the respiratory chain from multiple dehydrogenases linked to flavin adenine dinucleotide (FAD). These include enzymes of amino acid and choline metabolism in addition to the acyl-CoA dehydrogenases of ß-oxidation.

MAD deficiency has 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 of these patients have congenital anomalies (including large cystic kidneys, hypospadias and neuronal migration defects) and facial dysmorphism (low-set ears, high forehead and midfacial hypoplasia) [38]. The malformations resemble those seen in CPT II deficiency, but the pathogenesis is unknown. Most patients with neonatal-onset MAD deficiency 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 [39]. Cardiomyopathy is common in infants. Rarer problems include stridor and leukodystrophy [40]. 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 [39]. Rhabdomyolysis is rare, but there may be periods of rapidly worsening weakness. Many of the more mildly affected patients respond to riboflavin (► Section 13.6.3) [39].

Disorders of riboflavin transport can present with similar clinical and biochemical features. RFT2 (C20orf54) is thought to be the primary intestinal riboflavin transporter. RFT2 defects cause *Brown-Vialetto-van Laere syn-*

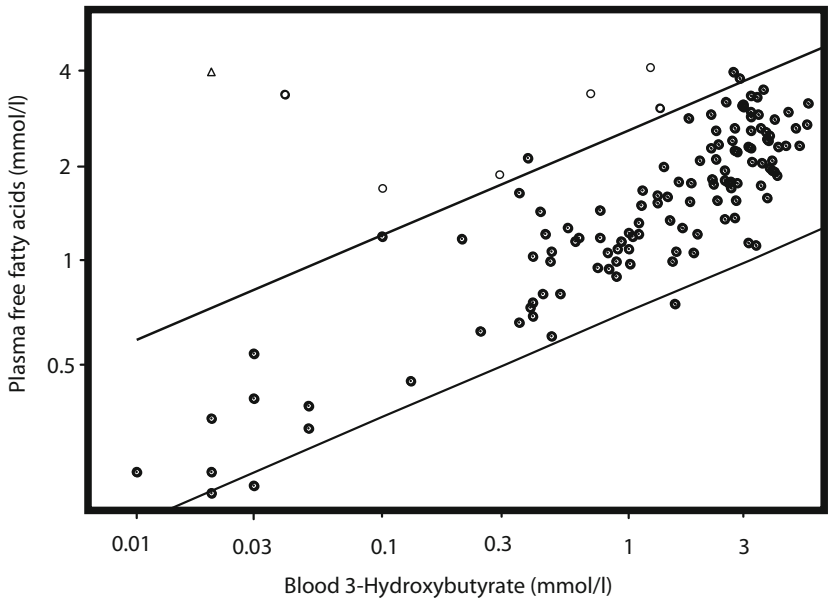


Fig. 13.2. The relationship between circulating free fatty acid and 3-hydroxybutyrate concentrations at the end of fasting tests. O established ß-oxidation defects; Δ patient with 3-hydroxy-3-methylglutaryl-CoA synthase deficiency; • other patients. The *solid lines* indicate the 95% predictive intervals for normal children (reproduced from [59] with permission from the BMJ publishing group)

drome, an autosomal recessive disorder that presents with hypotonia and respiratory failure in infancy or, later in life, with deafness and pontobulbar palsy; in the absence of deafness, it is known as Fazio-Londe disease. Blood acylcarnitines and urine organic acids suggest MAD deficiency, and treatment with riboflavin leads to clinical and biochemical improvement [41].

Impaired riboflavin transport has also been implicated in a *transient neonatal form* of MAD deficiency. Patients have presented within 24 h 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 mothers have had riboflavin deficiency, and one mother had a heterozygous mutation in the RFT1 riboflavin transporter (GPR172B) [42]. RFT1 is expressed in placenta as well as small intestine, and haplo-insufficiency may have caused severe riboflavin deficiency in the baby at birth.

13.3 Metabolic Derangement

Fasting hypoglycaemia is the classic metabolic disturbance in disorders of mitochondrial fatty acid oxidation and related metabolic pathways; this hypoglycaemia is due primarily to increased peripheral glucose consumption, though hepatic glucose output is also reduced under some conditions [43]. The hypoglycaemia is hypoketotic. Ketone bodies can be synthesised, particularly in medium- or short-chain FAODs [44] 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 (◘ Fig. 13.2). 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 [45]. Moderate hyperuricaemia is another frequent finding during acute attacks.

Accumulating long-chain acylcarnitines may be responsible for arrhythmias in these disorders [1, 46]. In LCHAD and MTP deficiencies, long-chain hydroxyacylcarnitine concentrations correlate with the severity of retinopathy [47], and they may cause both this and the peripheral neuropathy.

13.4 Genetics

All mitochondrial FAODs show an autosomal recessive pattern of inheritance, with the exception of transient neonatal MAD deficiency. Heterozygosity seldom causes problems except for mothers who are heterozygous for LCHAD deficiency, who may develop AFLP or HELLP syndrome when carrying an affected fetus. It has also been suggested that such symptoms as myopathy may occur in individuals who are heterozygous for more than

one defect of fatty acid oxidation or related pathways [48]; this proposal has been termed 'synergistic heterozygosity' and is supported by studies in mouse models [49].

There is molecular heterogeneity in all these disorders, but prevalent mutations have been identified in CPT I, CPT II, MCAD, SCAD and LCHAD deficiencies:

- *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% of babies are homozygous for c.1436C>T [8]. Though the mutation reduces CPT I activity to about 6% of 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.* In Caucasian patients with myopathic CPT II deficiency, the c.439C>T (p.S113L) mutation has a high prevalence, accounting for approximately 60% of mutant alleles [50].
- *MCAD Deficiency.* In Caucasian patients with MCAD deficiency, the c.985A>G mutation is even more prevalent: 80% of symptomatic patients and 60% of patients detected by screening are c.985A>G homozygotes [26, 27].
- *SCAD Deficiency.* There are two polymorphisms in the *SCAD* gene (c.625G>A and c.511C>T). In northern Europe, 6% of the general population have one of these variants on both alleles [31]. SCAD deficiency can be associated with these variants or with rare mutations.
- *LCHAD and MTP deficiency.* Most Caucasian patients are homozygous for the c.1528G>C mutation in the LCHAD domain of the α-subunit (74% in one international series [51]); this gives rise to isolated LCHAD deficiency. Patients with complete or partial deficiencies of all three 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 [22].

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 CPTII mutation and the c.848T>C VLCAD mutation) [15]. The latter is the commonest VLCAD mutation in caucasians and has only been found in mildly affected or asymptomatic patients. For other patients with VLCAD deficiency, it is easier to predict the clinical course from the enzyme activity.

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 MCAD mutations are, however, less likely to cause clinical problems. In particular, the c.199T>C mutation is associated with significant residual activity and appears to be benign: it accounts for about 6% of mutant alleles in screened populations, but it has never been found in a patient presenting clinically [26].

13.5 Diagnostic Tests

The investigation of a suspected FAOD starts with a search 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 nonspecifically 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. Many countries now have newborn screening programmes for FAODs (▶ Chapter 3).

13.5.1 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 blood spots on filter paper. Table 13.2 lists the typical abnormalities for different FAODs.

The diagnostic specificity can be increased by measuring the ratios of different acylcarnitines. For example, the C8/C10 acylcarnitine ratio can help to distinguish patients with MCAD deficiency from carriers or MAD-deficient patients. 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

Table 13.2. 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 acyl-carnitines, 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
SCHAD	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 the typical organic acids during acute illness; those in parentheses are mildly elevated. Organic acids are often normal during anabolism, except in some severe defects (e.g. MAD deficiency). *DCA*, C6-C10 saturated straight-chain dicarboxylic acids; *Variable DCA*, C6-C12 saturated and unsaturated straight-chain dicarboxylic acids. **In mild phenotypes, acylcarnitines can be completely normal during anabolism (e.g. VLCAD and MTP deficiencies) or even during catabolism (e.g. CPT II deficiency).
CACT, carnitine/acylcarnitine translocase; *CPT*, carnitine palmitoyltransferase; *CT*, carnitine transporter; *DCA*, dicarboxylic acid; *KB*, ketone bodies; *LCHAD*, long-chain 3-hydroxyacyl-CoA dehydrogenase, *MAD*, multiple acyl-CoA dehydrogenase; *MCAD*, medium-chain acyl-CoA dehydrogenase; *MTP*, mitochondrial trifunctional protein; *SCAD*, short-chain acyl-CoA dehydrogenase; *SCHAD*, short-chain 3-hydroxyacyl-CoA dehydrogenase; *VLCAD*, very-long-chain acyl-CoA dehydrogenase.

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 the levels seen in MAD deficiency.

Acylcarnitine analysis can be completely normal in patients with high residual enzyme activity, such as mild VLCAD 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 [52], but this is not entirely 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 acylcarnitines 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 [53]. Plasma free and total carnitine concentrations are usually less than 5 μmol/l in patients with carnitine transporter deficiency, though they may be higher in newborn babies [54]. Plasma free carnitine concentrations are often reduced in other FAODs owing to the accumulation of acylcarnitines, which competitively inhibit the carnitine transporter and increase the renal loss of carnitine [55].

In CPT I deficiency, plasma free carnitine concentrations are often (but not always) raised [56], allowing patients to be detected by newborn screening. Because

the defect prevents the formation of acylcarnitines, there is less competitive inhibition of the carnitine transporter than normal; this increases the reabsorption of carnitine from the urine and raises the plasma concentration.

- **Urinary Organic Acids and Acylglycines**

For many FAODs, organic acid analysis is normal when patients are well. During fasting or illness, however, medium-chain (and sometimes long-chain) dicarboxylic acids are elevated with little or no increase in ketone bodies. Dicarboxylic acids are formed whenever plasma free fatty acid concentrations are increased, by ß-oxidation in peroxisomes and ω-oxidation in microsomes, but normally they are accompanied by ketonuria. Dicarboxylic aciduria without ketonuria can also be seen in some respiratory chain defects.

More specific organic acids are seen in certain FAODs (◘ Table 13.2 and ► Chapter 4, Table 4.2). In severe defects, abnormal organic acids may be present even when the patient is well. Conversely, in myopathic CPT II deficiency, organic acids are normal even during acute crises. Patients with SCAD, MCKAT and mild MAD deficiencies can excrete large amounts of ketone bodies during illness, and even patients with MCAD deficiency may have significant ketonuria.

Abnormal glycine conjugates are found in some FAODs (◘ Table 13.2). Stable isotope-dilution mass spectrometry can reveal these even when patients are well [57].

13.5.2 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 [58]. The latter can be prepared from as little as 1-2 ml fresh 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, SCHAD) or specific under the assay conditions (e.g. VLCAD). Alternatively, the interfering enzyme can be inhibited or immunoprecipitated before performance of the assay (e.g. LCHAD, LCKAT). For some enzymes, such as MCAD, 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**

Increasingly often, molecular studies are used as an alternative to enzymology to confirm the diagnosis. This is satisfactory for well-defined mutations, such as the prevalent mutations seen in MCAD, LCHAD and CPT II deficiencies. The pathogenicity of other 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.

Where enzymology is the confirmatory test, mutation analysis may also be undertaken to define the disorder more precisely, allow carrier testing and simplify 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 incubation of fibroblasts or lymphocytes with fatty acids labelled with stable isotopes (^{2}H or ^{13}C) [59, 60]. This technique can identify most FAODs except carnitine transporter deficiency. CACT and severe CPT II deficiencies cannot be distinguished, and respiratory chain defects sometimes mimic FAODs [60].

Fatty acid oxidation flux is measured by incubating cells with radiolabelled fatty acids and collecting the oxidation products [61]. This is useful in evaluation of the severity of a disorder, but acylcarnitine profiling yields more diagnostic information.

13.5.3 Fasting Studies

In most FAODs, fasting leads to a fall in plasma glucose concentrations and a rise in free fatty acid concentrations, with an inappropriately small rise in ketone bodies (◘ Fig. 13.2). Samples collected during an episode of hypoglycaemia can, therefore, provide valuable information about its aetiology. In the past, fasting studies were a standard investigation if acute samples had not been obtained [62, 63]. For suspected FAODs, fasting studies have been supplanted by acylcarnitine analysis and in vitro studies, though they are still occasionally used in patients with unexplained hypoglycaemia (► Chapter 4). Fat loading tests are very seldom needed.

13.5.4 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 by means of enzyme assays.

13.5.5 Newborn Screening

Many countries now undertake newborn screening for FAODs, based on acylcarnitine analysis by TMS. The target conditions vary between countries, some only screening for MCAD deficiency (► Chapter 3).

13.6 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.

13.6.1 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 h until the patient starts to improve [64]. 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. 6-12 mg/kg/min, depending on age). Hypoglycaemia is a late event, and management should be started without delay, regardless of the blood glucose concentration.

13.6.2 Long-term Dietary Management

Prolonged fasting should be avoided in all FAODs to prevent acute metabolic decompensation. Frequent, regular feeds are recommended during the 1st 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 childhood, to reduce the risk of cardiomyopathy and long-term complications. These patients may be managed with continuous overnight tube feeding 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 the family history indicates a risk of MCAD deficiency, 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 independently 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 and 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 [17]; its use has also been associated with the resolution of renal tubular acidosis in CPT I deficiency [6]. Anecdotal evidence suggests that a bolus of MCT before exercise can prevent rhabdomyolysis in patients with myopathic VLCAD deficiency [65].

For symptomatic infants with long-chain FAODs, a formula maximally enriched with MCT is recommended and breast-feeding is avoided [66, 67]. After weaning, it has been suggested that MCT should provide 20% and long-chain fat, only 10% of the energy intake; in practice, this is hard to achieve in older patients, particularly as the long-chain fat has to include adequate essential fatty acids (4% of energy intake).

Newborn screening detects a number of mildly affected patients with long-chain FAODs. These patients 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 [67]. Dietary modification is recommended for all patients with LCHAD and MTP deficiencies, even if they are asymptomatic [66].

MCT is contraindicated in MCAD and MAD deficiencies because medium-chain fatty acids enter mitochondria rapidly, bypassing the normal regulation at CPT I, and cannot be oxidised. Instead, 3-hydroxybutyrate has been used in a few patients with moderately severe MAD deficiency. Sustained improvement was observed in two patients with severe cardiomyopathy and one patient with progressive leukodystrophy [40].

Triheptanoin has been substituted for MCT in a number of long-chain FAODs; there are reports of benefit in

three patients with VLCAD deficiency and seven patients with myopathic CPT II deficiency [68, 69]. 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 muscle, and that propionyl-CoA can replenish this loss. Controlled trials are needed to clarify the value of triheptanoin.

13.6.3 Drug Treatment

Carnitine treatment is very effective in patients with carnitine transporter deficiency. The cardiomyopathy and weakness resolve within a few months, and there are no further episodes of hypoglycaemia. With the usual dose of 100 mg/kg/day, plasma concentrations may reach the lower part of the normal range, but muscle carnitine concentrations remain less than 5% of normal [4].

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 [70]. Indeed, carnitine treatment may be harmful in long-chain FAODs, as it increases the concentrations of long-chain acylcarnitines, which are potentially arrhythmogenic [46].

Patients with mild MAD deficiency often respond to treatment with riboflavin (100 mg/day). Benefit is seen in some children who presented with hypoglycaemia, as well as in adults who presented with weakness [39]. Symptoms may resolve completely or there may be some residual weakness. Almost all these patients have mutations affecting ETFQO [39].

Bezafibrate is a particularly promising form of treatment for patients with myopathic CPT II or VLCAD deficiencies. The drug increases the expression of fatty acid oxidation enzymes by activating peroxisome proliferator-activated receptor (PPAR)α and PPARδ receptors. For mutations that leave significant residual activity, this can correct the deficiency in vitro [71]. When given to patients with mild CPT II deficiency, bezafibrate (200 mg three times daily) increased the enzyme activity in muscle and reduced the frequency of rhabdomyolysis [72].

13.6.4 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 creatine kinase (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 living on fat-modified diets. Hepatic steatosis and cardiomyopathy can be assessed by ultrasound. Ophthalmological and neurophysiological studies are needed in LCHAD and MTP deficiencies.

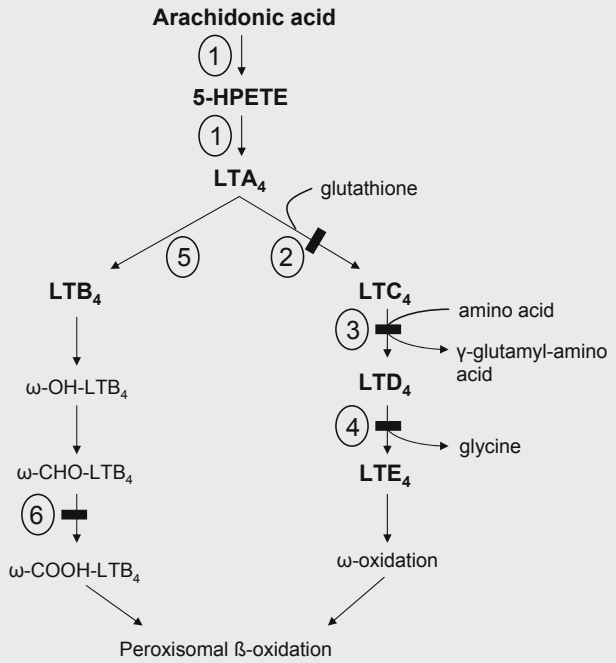
13.6.5 Prognosis

In the past, most FAODs involved a significant mortality during the presenting illness but had 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 the advent of screening programmes, approximately 4% of patients died in the first 72 h of life, and a further 5-7% died over the next 6 years [73]. After an episode of encephalopathy, about 7% of survivors have neuropsychological deficits. Newborn screening greatly reduces the morbidity and mortality, though it cannot eliminate early neonatal deaths [27]. 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 die in the presenting illness [19]. The prognosis is better with newborn screening, but many patients still have episodes of hypoglycaemia or rhabdomyolysis. There is also a high long-term risk of irreversible retinopathy or peripheral neuropathy. The neonatal-onset forms of CACT, CPT II, MTP and MAD deficiencies often present before screening results are available; they are almost invariably fatal within a few days or months.

Leukotriene Metabolism

Leukotrienes (LTs) are biologically active molecules derived from arachidonic acid. They are synthesised primarily by myeloid cells and act as mediators of inflammation; other effects include bronchoconstriction and contraction of intestinal smooth muscle. LTs are also formed in the brain, where they have neuromodulatory and neuroendocrine roles. The first two steps in LT synthesis (◘ Fig. 13.3) are catalysed by 5-lipoxygenase, in the presence of the 5-lipoxygenase activating protein. This forms LTA_4, which can either be hydrolysed to LTB_4 or conjugated with reduced glutathione to form LTC_4. Both LTB_4 and LTC_4 are exported from cells. LTC_4 is converted to LTD_4 and LTE_4 extracellularly, and all three are called cysteinyl LTs. LT degradation involves microsomal ω-oxidation followed by peroxisomal ß-oxidation: for LTB_4, microsomal fatty aldehyde dehydrogenase (FALDH) is required.



◘ **Fig. 13.3.** Pathway of leukotriene metabolism. *5-HPETE*, hydroperoxyeicosatetraenoic acid; *LT,* leukotriene; **1**, 5-lipoxygenase/FLAP (5-lipoxygenase activating protein); **2**, LTC_4 synthase; **3**, γ-glutamyl transpeptidase; **4**, membrane-bound dipeptidase; **5**, LTA_4 hydrolase; **6**, microsomal fatty aldehyde dehydrogenase. Enzyme defects are depicted by *solid bars*

13.7 Related Defects

13.7.1 Defects of Leukotriene (LT) Metabolism

Three primary defects in the synthesis of cysteinyl LTs have been identified. LTC_4 synthase deficiency is described here. γ-Glutamyl transpeptidase and membrane-bound dipeptidase deficiencies are included in ► Chapter 30. Secondary defects of LT elimination occur in Sjögren-Larsson syndrome (impaired ω-oxidation), Zellweger syndrome (impaired ß-oxidation) and Dubin-Johnson syndrome (impaired hepatobiliary elimination).

■ LTC_4 Synthase Deficiency

This has been reported in two unrelated infants from consanguineous pedigrees. There was intrauterine growth retardation, hypotonia with little spontaneous movement, severe psychomotor delay, microcephaly and failure to thrive [74]. Both patients died at 6 months of age. The cysteinyl-LTs were undetectable in plasma, urine and CSF and could not be synthesised by stimulated monocytes. Only supportive treatment is available.

13.7.2 Sjögren-Larsson Syndrome

This is an autosomal recessive disorder characterised by ichthyosis, spastic diplegia and mental retardation [75]. The prevalence is about 1:250,000, except in Northern Sweden, where it is 1:10,000 due to a founder effect.

Patients usually have dry, erythematous skin at birth and are often slightly preterm. Generalised scaling appears within 3 months, sparing the face. Later there may be lichenification on the neck, flexures and abdomen. There is severe pruritis (in contrast to other forms of ichthyosis) and often hypohydrosis. A spastic diplegia develops over the first 2 years; 50% of patients never walk, and many others require crutches. The spasticity slowly worsens over a few decades and causes contractures. Pseudobulbar dysarthria is common; spastic quadriplegia and seizures are rarer. Patients have mild to moderate mental retardation with a cheerful disposition and no regression; a few patients have normal intelligence [75]. Neuroimaging shows delayed myelination with a persistent mild deficit; proton MRS shows an abnormal lipid peak, which is probably due to accumulation of long-chain fatty alcohols or aldehydes. Most patients have a characteristic macular dystrophy with glistening yellow-white deposits and reduced macular pigment. This appears within the first 2 years, associated with photophobia and reduced visual acuity [75].

Sjögren-Larsson syndrome (SLS) results from a deficiency of the microsomal fatty aldehyde dehydrogenase (FALDH). FALDH can act independently or as part of the fatty alcohol: NAD oxidoreductase (FAO) complex. Substrates include aldehydes derived from fatty alcohols, ether glycerolipids, phytanic acid and fatty acids. FALDH is also involved in the inactivation of LTB_4 by ω-oxidation [76]. Consequently, patients with SLS have increased urine concentrations of LTB_4. They also have increased plasma concentrations of C16 and C18 fatty alcohols but measurement of these is difficult. The diagnosis can be confirmed by identifying mutations in the ALDH3A2 gene or by various techniques measuring FALD or FAO activity in fibroblasts or leukocytes [77]. Prenatal diagnosis is possible by mutation analysis or enzyme assay.

The pathogenesis of SLS is likely to be multifactorial and to involve reactive fatty aldehydes. LTB_4 concentrations are normal in CSF, suggesting that it does not cause the neuropathology. On the other hand, the pruritis may well be due to increased dermal LTB_4; reduced itching has been reported in several patients treated with zileuton, which inhibits the synthesis of leukotrienes [78]. Symptomatic treatment of the ichthiosis includes oral retinoids and topical emollients containing hydrating and keratolytic agents.

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Disorders of Ketogenesis and Ketolysis

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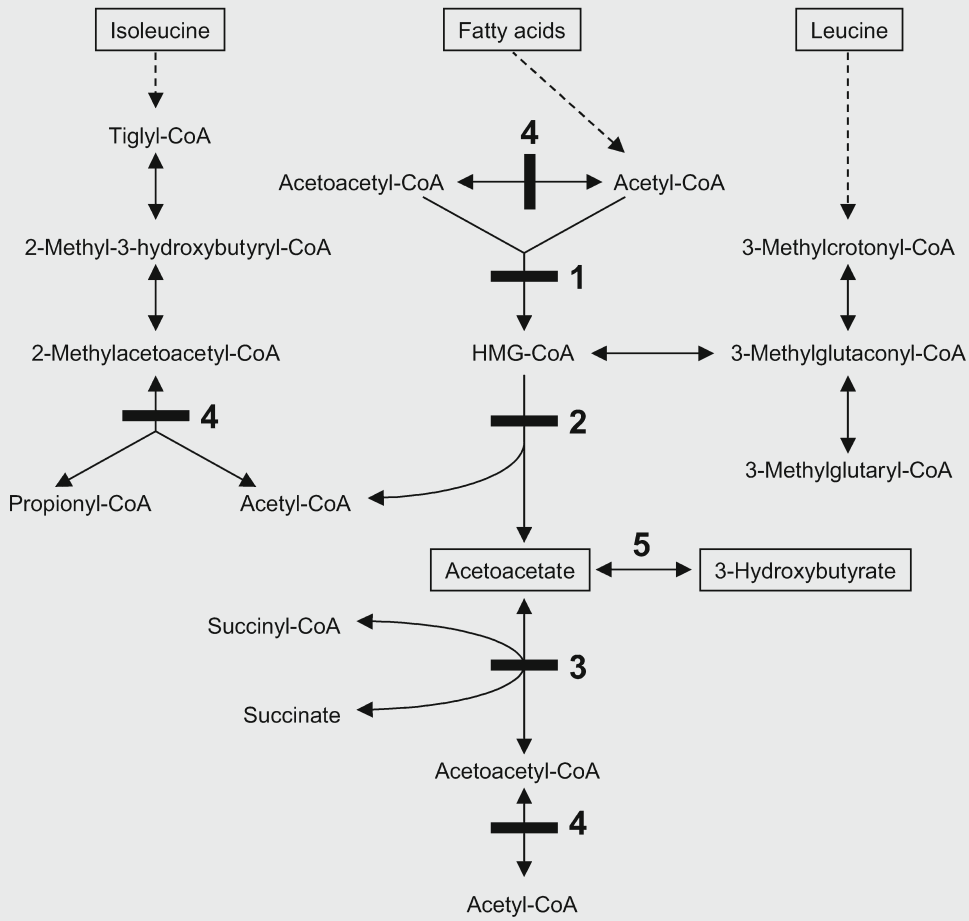
J.-M. Saudubray et. al (Eds.), *Inborn Metabolic Diseases,* DOI 10.1007/978-3-642-15720-2_14,

Ketogenesis and Ketolysis

During fasting, ketone bodies 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 ketone bodies, acetoacetate and 3-hydroxybutyrate, are maintained in equilibrium by 3-hydroxybutyrate dehydrogenase; acetone is formed from acetoacetate non-enzymatically and eliminated in breath. Ketone bodies are formed in liver mitochondria, predominantly from fatty acids, but also from certain amino acids, such as leucine. For use as fuel, ketone bodies are converted to acetyl-CoA in the mitochondria of extrahepatic tissues. One of the ketolytic enzymes, mitochondrial acetoacetyl-CoA thiolase, is also involved in the breakdown of isoleucine (◘ Fig. 14.1).



◘ **Fig. 14.1.** Biochemical pathways involving enzymes of ketogenesis and ketolysis. *HMG-CoA*, 3-hydroxy-3-methylglutaryl coenzyme A. **1**, Mitochondrial (m)HMG-CoA synthase; **2**, HMG-CoA lyase; **3**, succinyl-CoA 3-oxoacid CoA transferase (SCOT); **4**, mitochondrial acetoacetyl-CoA thiolase (T2); **5,** 3-hydroxybutyrate dehydrogenase. The enzyme defects discussed in this chapter are depicted by *solid bars* across the *arrows*

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. In defects of ketogenesis, 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. In defects of ketolysis, the clinical picture is dominated by severe ketoacidosis. This is often accompanied by decreased consciousness and dehydration.

14.1 Clinical Presentation

14.1.1 Mitochondrial 3-Hydroxy-3-Methylglutaryl-CoA Synthase Deficiency

Fewer than ten patients with mitochondrial 3-hydroxy-3-methylglutaryl-coenzyme A (mHMG-CoA) synthase deficiency (enzyme 1 in ◘ Fig. 14.1) have been reported. All presented with hypoglycaemia following infections with vomiting or poor feeding, between the ages of 9 months and 6 years [1-3]. At presentation, most patients were comatose and had hepatomegaly, which subsequently resolved. Blood lactate and ammonia concentrations were normal, and urine was negative for ketone bodies. All patients recovered promptly with intravenous glucose and none suffered long-term complications.

14.1.2 3-Hydroxy-3-Methylglutaryl-CoA Lyase Deficiency

Approximately 30% of patients with HMG-CoA lyase deficiency (enzyme 2 in ◘ Fig. 14.1) present by 5 days of age, after a short initial symptom-free period. Most of the other patients present later in the 1st year when they are fasted or suffer infections [4]. A few patients remain asymptomatic for a number of years [5], occasional ones presenting as adults [6].

Clinical features at presentation include vomiting, hypotonia and a reduced level of consciousness. Investigations show hypoglycaemia and acidosis. Ketone body levels are inappropriately low, but blood lactate concentrations may be markedly elevated, particularly in neonatal-onset cases [7]. Many patients have hyperammonaemia, hepatomegaly and abnormal liver function tests and, in the past, cases may have been misdiagnosed as Reye's syndrome. Pancreatitis and cardiomyopathy are recognised complications [8, 9], as in other branched-chain organic acidaemias. With appropriate treatment, most patients recover from their initial episode of metabolic decompensation. Unfortunately, a number suffer neurological sequelae, including epilepsy, intellectual handicap, hemiplegia or cerebral visual loss, particularly after neonatal hypoglycaemia [4, 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. There may also be abnormalities in the posterior limb of the internal capsule, the dentate nucleus and the pontine tegmentum [5]. The cause of the changes is unknown; myelination may be impaired because ketone bodies 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.

14.1.3 Succinyl-CoA 3-Oxoacid CoA Transferase Deficiency

The deficiency of succinyl-CoA 3-oxoacid CoA transferase (SCOT, also known as succinyl-CoA 3-ketoacid CoA transferase; enzyme 3 in ◘ Fig. 14.1) is characterised by recurrent episodes of severe ketoacidosis. Tachypnoea is often accompanied by hypotonia and lethargy. As for HMG-CoA lyase deficiency, 30% of patients become symptomatic within a few days of birth [10] and most of the others present later in the 1st year. A few patients have cardiomegaly at the time of presentation [11]. Blood glucose, lactate and ammonia concentrations are generally normal, though there have been reports of hypoglycaemia in neonates [10] and mild hyperglycaemia in older children [11]. Because ketosis and acidosis are common in sick children, SCOT deficiency enters the differential diagnosis for a large number of patients.

14.1.4 Mitochondrial Acetoacetyl-CoA Thiolase Deficiency

Recurrent episodes of ketoacidosis also occur in patients with a deficiency of mitochondrial acetoacetyl-CoA thiolase (also known as 2-methyl-acetoacetyl-CoA thiolase and as β-ketothiolase, abbreviated T2; enzyme 4 in ◘ Fig. 14.1). Neonatal onset is rare [12]. Most patients present during the first 2 years of life, but some remain asymptomatic into adulthood [13].

Episodes of decompensation generally start with tachypnoea and vomiting, followed by dehydration and a falling level of consciousness [12]. A few patients have seizures. Investigations show a severe metabolic acidosis with ketonuria. Blood glucose, lactate and ammonia concentrations are normal in most cases, but there may be hypo- or hyperglycaemia [11, 12]. The high acetoacetate levels in blood and urine can cause screening tests for salicylate to give false-positive results [14]. Cardiomyopathy is a rare complication [15]. Most patients make a full recovery, but some have mental retardation, ataxia or dystonia with abnormalities in the basal ganglia on MRI [16]. A few patients have developmental delay before their first episode of ketoacidosis [16].

14.2 Metabolic Derangement

Ketone bodies are synthesised in hepatic mitochondria, primarily using acetyl-CoA derived from fatty acid oxidation (◘ Fig. 14.1). mHMG-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, mHMG-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 ketone bodies leads to excessive glucose consumption and hypoglycaemia.

Ketone body 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. 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 by converting acetyl-CoA to acetoacetyl-CoA. T2 also cleaves 2-methylacetoacetyl-CoA in the degradation pathway for isoleucine. Patients with T2 deficiency present with ketoacidosis, implying that the enzyme is more crucial in ketolysis than in ketogenesis; they also excrete intermediates of isoleucine catabolism.

14.3 Genetics

All four disorders are inherited as autosomal recessive traits. Their prevalence is unknown, but HMG-CoA lyase deficiency is relatively common in Saudi Arabia [7]. Mutations have been identified in patients with each of the four disorders [2, 12, 17, 18]. Two common mutations have been found in HMG-CoA lyase deficiency, one in the Saudi population (c.122G>A) and the other in patients from the Iberian peninsula (c.109G>A) [18]. Common mutations have not been identified in the other disorders. The genotype correlates poorly with the clinical phenotype, which depends on exposure to environmental stress [12, 18].

14.4 Diagnostic Tests

HMG-CoA lyase and T2 deficiencies are usually identified by detecting abnormal urinary organic acids. They are also associated with abnormal acylcarnitines and, in some countries, they are targets for newborn screening. The organic acid and acylcarnitine abnormalities are sometimes hard to detect in T2 deficiency. Recognition of the other defects is more difficult, and it is likely that many cases remain undiagnosed.

14.4.1 Mitochondrial 3-Hydroxy-3-Methylglutaryl-CoA Synthase Deficiency

This diagnosis should be suspected when there is grossly impaired ketogenesis in vivo but normal fatty acid oxidation flux in vitro (▶ Chapters 3, 13). Fasting leads to massive dicarboxylic aciduria (saturated, unsaturated and 3-hydroxy-compounds) with low levels of ketone bodies [2, 3]. Blood acylcarnitine analysis is normal when patients are well, but acetylcarnitine may be raised during illness [3]. Measurement of enzyme activity requires a liver biopsy because it is only expressed at high levels in liver and testis. Moreover, enzyme assays are complicated by a cytoplasmic isoenzyme, involved in cholesterol synthesis, which accounts for approximately 10% of total activity. Enzymology has not been undertaken in recent patients, and the diagnosis has been confirmed by mutation analysis [2, 3].

14.4.2 3-Hydroxy-3-Methylglutaryl-CoA Lyase Deficiency

Patients with this condition excrete increased quantities of 3-hydroxy-3-methylglutaric, 3-hydroxyisovaleric, 3-methylglutaconic and 3-methylglutaric acids (◘ Fig. 14.1); 3-methylcrotonylglycine may also be present [4]. It is important to confirm the diagnosis enzymologically since a similar pattern of urinary organic acids has been found in patients with normal HMG-CoA lyase activity [19]. Assays can be undertaken on leukocytes or cultured fibroblasts as well as liver. HMG-CoA lyase deficiency leads to raised blood 3-hydroxyisovalerylcarnitine concentrations that can be detected by tandem mass spectrometry. It is included in the expanded newborn screening programmes for several countries, including the USA. Cases need to be distinguished from four other inborn errors that cause increased 3-hydroxyisovalerylcarnitine or 2-methyl-3-hydroxybutyrylcarnitine, which has the same mass (3-methylcrotonyl-CoA carboxylase, 3-methylglutaconyl-CoA hydratase, 2-methyl-3-hydroxybutyryl-CoA dehydrogenase and T2 deficiencies).

14.4.3 Succinyl-CoA 3-Oxoacid CoA Transferase Deficiency

Most patients have persistent ketonuria, even in the fed state, with a plasma concentration of ketone bodies (ac-

etoacetate plus 3-hydroxybutyrate) above 0.2 mmol/l [11]. Patients with mild mutations, however, may not have ketonuria when they are well [20]. If a diagnostic fast is undertaken there is an excessive rise in blood ketone body levels, sometimes to over 10 mmol/l, without hypoglycaemia [11, 20]. Urinary organic acid analysis reveals high concentrations of 3-hydroxybutyrate, acetoacetate and sometimes 3-hydroxyisovalerate, but no specific abnormalities. The diagnosis must, therefore, be confirmed by enzymology, which can be undertaken on lymphocytes or cultured fibroblasts. Even in patients with two null mutations, assays generally show 20–35% apparent residual activity, which is due to the consumption of substrate by other enzymes [17].

14.4.4 Mitochondrial Acetoacetyl-CoA Thiolase Deficiency

Patients with T2 deficiency typically excrete increased amounts of 2-methylacetoacetate, 2-methyl-3-hydroxybutyric acid and tiglylglycine (◘ Fig. 14.1). However, 2-methylacetoacetate may not be detected because it is unstable and tiglylglycine and 2-methyl-3-hydroxybutyric acid may not be increased in unstressed patients with mild mutations [21]. An isoleucine load may be needed to demonstrate the abnormalities. 2-Methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency causes a similar pattern of organic acids, but 2-methyacetoacetate is not excreted (► Chapter 19). It is, therefore, important to confirm the diagnosis by mutation analysis or enzyme assay in fibroblasts. Assays are complicated by the presence of three other thiolases that act on acetoacetyl-CoA (cytosolic and peroxisomal acetoacetyl-CoA thiolases and mitochondrial medium chain 3-ketoacyl-CoA thiolase). 2-Methylacetoacetyl-CoA is a specific substrate for T2 but it is difficult to synthesise [22]. One solution is to measure acetoacetyl-CoA thiolysis in the presence and absence of potassium, which enhances the activity of T2 but not the other enzymes [14].

Blood acylcarnitine analysis in T2 deficiency generally shows raised 2-methyl-3-hydroxybutyrylcarnitine and tiglylcarnitine, but the concentrations are borderline in unstressed patients with mild mutations [21]. T2 deficiency is included in some expanded newborn screening programmes, but a few patients are missed [23] and several other inborn errors need to be distinguished (► Section 14.4.2).

14.4.5 Prenatal Diagnosis

Prenatal diagnosis is possible using molecular techniques in families where the mutations are known [24, 25]. Alternatively, prenatal diagnosis can be performed by enzyme assay in chorionic villi or cultured amniocytes [24, 25]. This is not possible for mHMG-CoA synthase, and some authorities prefer not to use chorionic villi for SCOT [26]. Organic acid analysis of amniotic fluid is a third method of prenatal diagnosis for HMG-CoA lyase deficiency [24].

14.5 Treatment and Prognosis

All 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 not tolerated or if a patient with a ketolysis disorder develops moderate or heavy ketonuria. In hospital, patients require an intravenous infusion of glucose. Acidosis is common in HMG-CoA lyase deficiency and, particularly, in the ketolysis disorders. An intravenous infusion of bicarbonate is needed if the acidosis is severe (pH<7.1), and it may be given in milder acidosis but electrolytes must be monitored frequently: there is a risk of severe and potentially fatal hypernatraemia. Extra fluids may be needed to correct dehydration, which is common in the ketolysis disorders.

A moderate protein restriction is usually recommended in HMG-CoA lyase, SCOT and T2 deficiencies, because these enzymes are directly or indirectly involved in amino acid catabolism [4, 11, 12]. A low-fat diet has also been recommended [27]. Protein and fat should certainly be avoided during illness. At other times, however, dietary restriction is unnecessary in some patients [13, 17]. Carnitine supplements are often given if serum levels are low, though their value is unproven.

Patients with these disorders can die or suffer irreversible neurological damage during episodes of metabolic decompensation. Outcomes have been least good for neonatal-onset cases of HMG-CoA lyase deficiency, such as those from Saudi Arabia [7]. Once the diagnosis has been made, the outlook is much improved. Patients become more stable with age, particularly those with ketolysis defects [12]. One patient with T2 deficiency had a healthy child following an uncomplicated pregnancy [28].

14.6 Cytosolic Acetoacetyl-CoA Thiolase Deficiency

Cytosolic acetoacetyl-CoA thiolase (CAT) is primarily involved in the synthesis of isoprenoid compounds, such as cholesterol, rather than ketone body metabolism. CAT

deficiency has been reported in two patients with mental retardation and persistent ketosis [29]. Mutation analysis has not been undertaken, and the diagnoses remain uncertain.

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Defects of the Respiratory Chain

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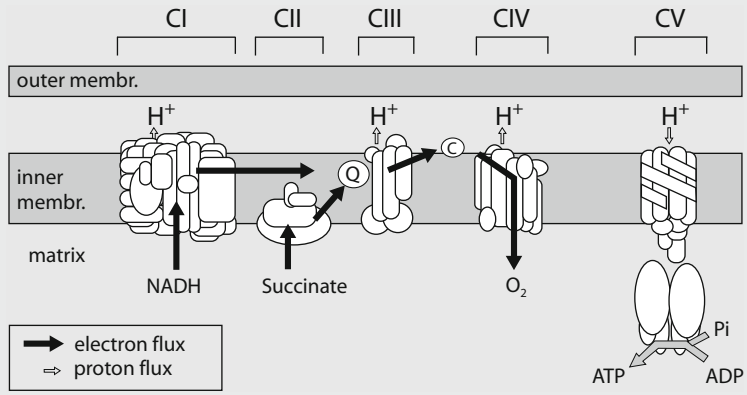
The Respiratory Chain

The respiratory chain (◘ Fig. 15.1) is composed of five functional units or complexes embedded in the inner mitochondrial membrane [1]. *Complex I* [CI, reduced nicotinamide adenine dinucleotide (NADH)-coenzyme Q (CoQ) reductase] carries reducing equivalents from NADH to CoQ and consists of 25-28 different polypeptides, 7 of which are encoded by mitochondrial DNA. *Complex II* (CII, succinate-CoQ reductase) carries reducing equivalents from reduced flavin adenine dinucleotide ($FADH_2$) to CoQ and contains five polypeptides, including the flavin adenine dinucleotide-dependent succinate dehydrogenase and a few non-haem iron-sulfur centres. *Complex III* (CIII, reduced CoQ-cytochrome-c reductase) carries electrons from CoQ to cytochrome c and contains 11 subunits. *Complex IV* (CIV, cytochrome-c oxidase) catalyses the transfer of reducing equivalents from cytochrome c to molecular oxygen. It is composed of two cytochromes (a and a_3), two copper atoms, and 13 different protein subunits.

The respiratory chain catalyses the oxidation of fuel molecules by oxygen and the concomitant energy transduction into ATP. During the oxidation process, electrons are transferred to oxygen via the energy-transducing complexes: complexes I, III and IV for succinate and complexes III and IV for $FADH_2$ derived from the β-oxidation pathway via the electron-transfer flavoprotein (ETF) and the ETF-CoQ oxidoreductase system. CoQ (a lipoidal quinone) and cytochrome c (a low-molecular-weight haemoprotein) act as shuttles between the complexes.

The flux of electrons is coupled to the translocation of protons (H^+) into the intermembrane space at three coupling sites (complexes I, III and IV). This creates a transmembrane gradient. *Complex V* (CV, ATP synthase) allows protons to flow back into the mitochondrial matrix and uses the released energy to synthesise ATP. Three molecules of ATP are generated for each molecule of NADH oxidised.



◘ **Fig. 15.1.** The mitochondrial respiratory chain. *ADP*, Adenosine diphosphate; *ATP*, adenosine triphosphate; *c*, cytochrome c; *CI*, complex I (NADH coenzyme-Q reductase); *CII*, complex II (succinate-coenzyme-Q reductase); *CIII*, complex III (reducedcoenzyme- Q-cytochrome-c reductase); *CIV*, complex IV (cytochrome- c oxidase); *CV*, complex V (ATP synthase); *NADH*, reduced nicotinamide adenine dinucleotide; *Pi*, inorganic phosphate; *Q*, coenzyme Q

15

Respiratory chain deficiencies have long been regarded as neuromuscular diseases only. However, *oxidative phosphorylation* (i.e. ATP synthesis by the respiratory chain) is not restricted to the neuromuscular system, but proceeds in all cells that contain mitochondria. Most nonneuromuscular organs and tissues are, therefore, also dependent upon mitochondrial energy supply. Owing to the twofold genetic origin of respiratory enzymes (nuclear DNA and mitochondrial [mtDNA]), a respiratory chain deficiency can theoretically give rise to any symptom in any organ or tissue at any age and with any mode of inheritance.

The diagnosis of a respiratory chain deficiency is difficult to consider initially when only one abnormal symptom is present. In contrast, this diagnosis is easier to consider when two or more seemingly unrelated symptoms are observed. The treatment, mainly dietetic, does not markedly influence the usually unfavourable course of the disease.

15.1 Clinical Presentation

Owing to the ubiquitous nature of oxidative phosphorylation, a defect of the mitochondrial respiratory chain should be considered in patients presenting (1) with an unexplained association of neuromuscular and/or non-neuromuscular symptoms, (2) with a rapidly progressive course, and (3) with symptoms involving seemingly unrelated organs or tissues. The disease may begin at virtually any age. ◘ Table 15.1 summarises the most frequently observed symptoms. Whatever the age of onset and the

Table 15.1. The most frequently observed symptoms in defects of the respiratory chain

Neonatal period (0–1 month)
- Central nervous system
  - Iterative apnoea, lethargy, drowsiness, near-miss
  - Limb and trunk hypotonia
  - Congenital lactic acidosis
  - Ketoacidotic coma
- Muscle
  - Macroglossia
  - Myopathic presentation
  - Muscular atrophy, hypotonia
  - Stiffness, hypertonia
  - Recurrent myoglobinuria
  - Poor head control, poor spontaneous movement
- Liver
  - Hepatic failure, liver enlargement
- Heart
  - Hypertrophic cardiomyopathy (concentric)
- Kidney
  - Proximal tubulopathy (De Toni-Debré-Fanconi syndrome)

Infancy (1 month–2 years)
- Central nervous system
  - Recurrent apnoeas, near-miss
  - Recurrent ketoacidotic coma
  - Poor head control, limb spasticity
  - Psychomotor regression, mental retardation
  - Cerebellar ataxia
  - Stroke-like episodes
  - Myoclonus, generalised seizures
  - Subacute necrotising encephalomyopathy (Leigh syndrome)
  - Progressive infantile poliodystrophy (Alpers syndrome)
- Muscle
  - Myopathic features
  - Muscular atrophy
  - Limb weakness, hypotonia
  - Myalgia, exercise intolerance
  - Recurrent myoglobinuria
- Liver
  - Progressive liver enlargement
  - Hepatocellular dysfunction
  - Valproate-induced hepatic failure
- Heart
  - Hypertrophic cardiomyopathy (concentric)
- Kidney
  - Proximal tubulopathy (De Toni-Debré-Fanconi syndrome)
  - Tubulo-interstitial nephritis (mimicking nephronophtisis)
  - Nephrotic syndrome
  - Renal failure
  - Haemolytic uraemic syndrome
- Gut
  - Recurrent vomiting
  - Chronic diarrhoea, villous atrophy
  - Exocrine pancreatic dysfunction
  - Failure to thrive
  - Chronic interstitial pseudo-obstruction
- Endocrine
  - Short stature, retarded skeletal maturation
  - Recurrent hypoglycaemia
  - Multiple hormone deficiency
- Bone marrow
  - Sideroblastic anaemia
  - Neutropenia, thrombopenia
  - Myelodysplastic syndrome, dyserythropoiesis
- Ear
  - Hearing loss
  - Sensorineural deafness (brain stem or cochlear origin)
- Eye
  - Optic atrophy
  - Diplopia
  - Progressive external ophthalmoplegia
  - Limitation of eye movements (all directions, upgaze)
  - Salt-and-pepper retinopathy, pigmentary retinal degeneration
  - Lid ptosis
  - Cataract
- Skin
  - Mottled pigmentation of photo-exposed areas
  - Trichothiodystrophy
  - Dry, thick and brittle hair

Childhood (>2 years) and adulthood
- Central nervous system
  - Myoclonus
  - Seizures (generalised, focal, drop attacks, photosensitivity, tonicoclonus)
  - Cerebellar ataxia
  - Spasticity
  - Psychomotor regression, dementia, mental retardation
  - 'Stroke-like' episodes
  - Hemicranial headache, migraine
  - Recurrent hemiparesis, cortical blindness or hemianopsia
  - Leukodystrophy, cortical atrophy
  - Peripheral neuropathy
- Muscle
  - Progressive myopathy
  - Limb weakness (proximal)
  - Myalgia, exercise intolerance
  - Recurrent myoglobinuria
- Heart
  - Concentric hypertrophic or dilated cardiomyopathy
  - Different types of heart block
- Endocrine
  - Diabetes mellitus (insulin- and non-insulin-dependent)
  - Growth-hormone deficiency
  - Hypoparathyroidism
  - Hypothyroidism
  - Adrenocorticotrophin deficiency
  - Hyperaldosteronism
  - Infertility (ovarian failure or hypothalamic dysfunction)
- Eye
  - Lid ptosis
  - Diplopia
  - Progressive external ophthalmoplegia
  - Limitation of eye movements (all directions, upgaze)
  - 'Salt-and-pepper' retinopathy, pigmentary retinal degeneration
  - Cataract, corneal opacities
  - Leber hereditary optic neuroretinopathy
- Ear
  - Sensorineural deafness
  - Aminoglycoside-induced ototoxicity (maternally inherited)

presenting symptom, the major feature is the increasing number of tissues affected in the course of the disease. This progressive organ involvement is constant, and the central nervous system is almost consistently involved in the late stage of the disease.

While the initial symptoms usually persist and gradually worsen, they may occasionally improve or even disappear as other organs become involved. This is particularly true for bone marrow and gut. Indeed, remarkable remissions of pancytopenia or watery diarrhoea have been reported in infants who later developed other organ involvements. Moreover, several patients whose disease apparently started in childhood or adulthood were retrospectively shown to have experienced symptoms (transient sideroblastic anaemia, neutropenia, chronic watery diarrhoea, or failure to thrive) of unexplained origin in early infancy. Similarly, a benign reversible infantile myopathy with hypotonia, weakness, macroglossia, respiratory distress and spontaneous remission within 12 years has been described.

Certain clinical features or associations are more frequent at certain ages and have occasionally been identified as distinct entities, suggesting that these associations are not fortuitous. However, considerable overlap in clinical features leads to difficulties in the classification of many patients, and the nature, clinical course and severity of symptoms vary among (and even within) affected individuals. It is more useful to bear in mind that the diagnosis of respiratory chain deficiency should be considered regardless of the age of onset and the nature of the presenting symptom when a patient presents with an unexplained association of signs with a progressive course involving seemingly unrelated organs or tissues. The nonexhaustive list of clinical profiles illustrates the diversity of presentations (◘ Table 15.1).

15.1.1 Fetuses

Intrauterine growth retardation (below 3rd percentile for gestational age), either in isolation or associated with otherwise unexplained antenatal anomalies, is retrospectively detected in 20% of respiratory enzyme chain-deficient paediatric patients [2]. Antenatal anomalies are usually multiple and include polyhydramnios, oligoamnios, arthrogryposis, decreased fetal movements, ventricular septal defects, hypertrophic cardiomyopathy, vertebral and limb defects or other visceral anomalies (VACTERL association). At variance with a number of metabolic diseases that have a symptom-free period, respiratory chain deficiency may have an antenatal expression related to the time course of the disease gene expression in the embryofetal period.

15.1.2 Neonates

In the neonate (age less than 1 month), the following clinical profiles are seen:

- Ketoacidotic coma with recurrent apneas, seizures, severe hypotonia, macroglossia, liver enlargement, and proximal tubulopathy, with or without a symptom-free period.
- Severe sideroblastic anaemia (with or without hydrops fetalis), with neutropenia, thrombocytopenia, and exocrine pancreatic dysfunction of unexplained origin (Pearson marrow-pancreas syndrome) [3].
- Concentric hypertrophic cardiomyopathy and muscle weakness with an early onset and a rapidly progressive course.
- Concentric hypertrophic cardiomyopathy with profound central neutropenia and myopathic features in males (Barth syndrome, ► Chapter 35).
- Hepatic failure with lethargy, hypotonia and proximal tubulopathy with or without mtDNA depletion [4].

15.1.3 Infants

In infancy (1 month to 2 years), the clinical profiles include the following:

- Failure to thrive, with or without chronic watery diarrhoea and villous atrophy; unresponsiveness to gluten-free and cow's milk protein-free diet.
- Recurrent episodes of acute myoglobinuria, hypertonia, muscle stiffness and elevated plasma levels of enzymes unexplained by an inborn error of glycolysis, glycogenolysis, fatty acid oxidation, lipin-1 deficiency or muscular dystrophy.
- Proximal tubulopathy (de Toni-Debré-Fanconi syndrome) with recurrent episodes of watery diarrhoea, rickets and mottled pigmentation of photo-exposed areas.
- A tubulo-interstitial nephritis mimicking nephronophtisis, with the subsequent development of renal failure and encephalomyopathy with leukodystrophy.
- Growth retardation, aminoaciduria, cholestasis, iron overload lactic acidosis and early death (GRACILE syndrome) [5].
- Sensorineural hearing loss and pili torti (Bjornstad syndrome) [6].
- Severe trunk and limb dwarfism unresponsive to growth-hormone administration, with subsequent hypertrophic cardiomyopathy, sensorineural deafness, and retinitis pigmentosa.

- Early-onset insulin-dependent diabetes mellitus with diabetes insipidus, optic atrophy and deafness (Wolfram syndrome) [7].
- Myopathy, lactic acidosis, and sideroblastic anaemia (MLASA syndrome) [8, 9].
- Rapidly progressive encephalomyopathy with hypotonia, poor sucking, weak crying, poor head control, cerebellar ataxia, pyramidal syndrome, psychomotor regression, developmental delay, muscle weakness, and respiratory insufficiency; occasionally associated with proximal tubulopathy and/or hypertrophic cardiomyopathy.
- Subacute necrotising encephalomyopathy (Leigh's disease). This is a devastating encephalopathy characterised by recurrent attacks of psychomotor regression with pyramidal and extrapyramidal symptoms, leukodystrophy, and brain stem dysfunction (respiratory abnormalities). The pathological hallmark consists of focal, symmetrical, and necrotic lesions in the thalamus, the brain stem, and the posterior columns of the spinal cord. Microscopically, these spongiform lesions show demyelination, vascular proliferation, and astrocytosis.

15.1.4 Children and Adults

In childhood (above 2 years) and adulthood, the neuromuscular presentation is the most frequent:

- Muscle weakness with myalgia and exercise intolerance, with or without progressive external ophthalmoplegia.
- Ataxia, cerebellar atrophy, muscle weakness, seizures and mental retardation.
- Progressive sclerosing poliodystrophy (Alpers disease) associated with hepatic failure.
- Encephalomyopathy with myoclonus, ataxia, hearing loss, muscle weakness and generalised seizures (myoclonus epilepsy, ragged red fibres, MERRF).
- Progressive external ophthalmoplegia (PEO) ranging in severity from pure ocular myopathy to Kearns-Sayre syndrome (KSS). KSS is a multisystem disorder characterised by the triad of (1) onset before age 20 years, (2) PEO and (3) pigmentary retinal degeneration, plus at least one of the following: complete heart block, cerebrospinal fluid (CSF) protein levels above 100 mg/dl and cerebellar ataxia.
- Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS). This syndrome is characterised by onset in childhood, with intermittent hemicranial headache, vomiting, proximal limb weakness, and recurrent neurological deficit resembling strokes (hemiparesis, cortical blindness, hemianopsia), lactic acidosis, and ragged red fibres (RRFs) in the muscle biopsy. Brain MRI shows low-density areas (usually posterior), which may occur in both white and grey matter but do not always correlate with the clinical symptoms or the vascular territories. The pathogenesis of stroke-like episodes in MELAS has been ascribed to either cerebral blood-flow disruption or acute metabolic decompensation in biochemically deficient areas of the brain.
- Leber's hereditary optic neuroretinopathy (LHON). This disease is associated with rapid loss of bilateral central vision due to optic nerve death. Cardiac dysrythmia is frequently associated with the disease, but no evidence of skeletal muscle pathology or gross structural mitochondrial abnormality has been documented. The median age of vision loss is 20-24 years, but it can occur at any age between adolescence and late adulthood. Expression among maternally related individuals is variable, and more males are affected. Kjer's autosomal dominant optic atrophy is also caused by a mitochondrial dysfunction in the dynamin-related protein OPA.
- Neurogenic muscle weakness, ataxia, retinitis pigmentosa (NARP) and variable sensory neuropathy with seizures and mental retardation or dementia.
- Mitochondrial myopathy, PEO, ptosis, peripheral neuropathy, diffuse leuko-encephalopathy and gastrointestinal dysmotility manifesting as intermittent diarrhoea and intestinal pseudo-obstruction (myoneurogastrointestinal encephalopathy, MNGIE).
- Progressive multisystem failure with encephalopathy, myopathy, peripheral neuropathy and renal failure.

15.2 Metabolic Derangement

As the respiratory chain transfers electrons to oxygen, a disorder of oxidative phosphorylation should result in (1) an increase in the concentration of reducing equivalents in both mitochondria and cytoplasm and (2) the functional impairment of the citric acid cycle, resulting from the excess of reduced nicotinamide adenine dinucleotide (NADH) and the lack of nicotinamide adenine dinucleotide (NAD). Therefore, an increase in the ketone body (3-hydroxybutyrate/acetoacetate) and lactate/pyruvate (L/P) molar ratios with a secondary elevation of blood lactate might be expected in the plasma of affected individuals. This is particularly true in the postabsorptive period, when more NAD is required to oxidise glycolytic substrates adequately.

Similarly, as a consequence of the functional impairment of the citric acid cycle, ketone body synthesis increases after meals, because of the channelling of acetyl-coenzyme A (CoA) towards ketogenesis. The elevation of the total level of ketone bodies in a fed individual is paradoxical, as it should normally decrease after meals, due to insulin release (paradoxical hyperketonaemia).

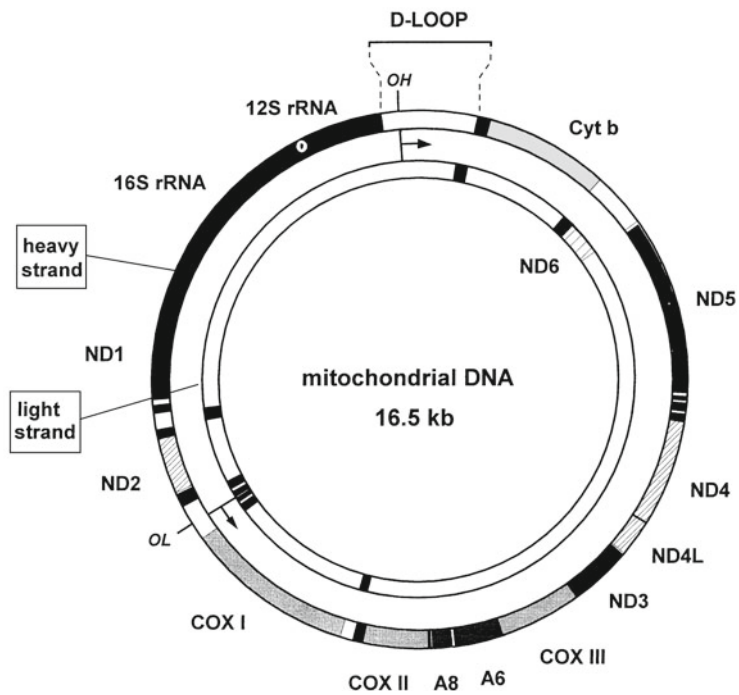
The position of the block might differentially alter the metabolic profile of the patient. At the level of CI, it impairs the oxidation of the 30 mol of NADH formed in the citric acid cycle. In theory at least, oxidation of reduced flavin adenine dinucleotide ($FADH_2$) derived from succinate-producing substrates (methionine, threonine, valine, isoleucine and odd-numbered fatty acids) should not be altered, because it is mediated by CII. Similarly, oxidation of $FADH_2$ derived from the first reaction of the β-oxidation pathway should occur normally, because it is mediated by the electron transfer flavoprotein coenzyme-Q-reductase system. However, CII deficiency should not markedly alter the redox status of affected individuals fed a carbohydrate-rich diet. A block at the level of CIII should impair the oxidation of both NAD-linked and FAD-linked substrates. Finally, given the crucial role of CIV in the respiratory chain, it is not surprising that severe defects of cytochrome c oxidase activity cause severe lactic acidosis and markedly alter redox status in plasma.

15.3 Genetics

Any mode of inheritance may be observed in mitochondrial diseases: autosomal recessive, dominant, X-linked, maternal, or sporadic. This variability is due to the high number of genes that encode the respiratory chain proteins, 13 of which are located in the mitochondria. mtDNA encodes seven polypeptides of CI, one of CIII (the apoprotein of cytochrome b), three of CIV, and two of CV. The mtDNA molecules are small (16.5 kb), double-stranded and circular, and they contain no introns (◘ Fig. 15.2). mtDNA has a number of unique genetic features:

- It is maternally inherited, and its mutations are, therefore, either de novo or transmitted by the mother.
- It has a very high mutation rate, involving both nucleotide substitutions and insertions/deletions.

During cell division, mitochondria are randomly partitioned into daughter cells. This means that, if normal and mutant mtDNA molecules are present in the mother's cells (heteroplasmy), some lineages will have only abnormal mtDNA (homoplasmy), others will have only normal mtDNA (wild type) and still others will have both normal and abnormal mtDNA. In these last cells, the phenotype will reflect the proportion of abnormal mtDNA.



◘ **Fig. 15.2.** Map of the mitochondrial genome. Regions encoding cytochrome b (*cyt b*), various subunits of reduced nicotinamide adenine dinucleotide-coenzyme Q reductase (*ND*), cytochrome oxidase (*COX*), and adenosine triphosphatase (*A*) and rRNAs are indicated. Replication of the heavy strand starts in the displacement (*D*) loop at the heavy-strand origin (*OH*) and that of the light strand, at OL

The nuclear genome encodes a number of proteins involved in mtDNA replication, transcription, translation, fusion and fission, mitochondrial transport (of ions, nucleotides, amino acids, metals and vitamins), co-enzyme Q synthesis, and components of the mitochondrial ribosome, multiple structural, chaperone and assembly proteins, and the remaining catalytic subunits of the respiratory enzyme complexes (other than the 13 mtDNA-encoded subunits).

15.3.1 Mitochondrial DNA Mutations

Pathological alterations of mtDNA fall into three major categories: point mutations, deletion-duplications and copy-number mutations (depletions). In most cases, mtDNA mutations are heteroplasmic, as both normal and mutant mtDNA are present. The phenotype is a reflection of the proportion of mutant mtDNA molecules and the extent to which the cell type relies on mitochondrial function. Point mutations include amino acid substitutions and protein synthesis mutations (tRNA, rRNA).

▪ Mitochondrial Protein Synthesis Mutations

The A3243G mutation in the *tRNALeu* gene is responsible for MELAS [10]. The A3243G mutation can also cause maternally inherited diabetes mellitus (either isolated or associated with hearing loss, cardiomyopathy, headache or renal disease) [11]. It is generally assumed that 1.5% of cases of diabetes in Europe are due to mtDNA mutations (including the A3243G mutation) and perhaps as many as 5% of cases in East Asia [12].

The A8344G mutation in the mt *tRNALys* gene accounts for most cases of MERRF [13]. Several other mutations of either tRNA or rRNA genes have been reported.

▪ Mutations in Protein-coding Genes

Mutations in mtDNA-encoded subunits are frequent causes of respiratory chain deficiency. Systematic mtDNA sequencing in our series has shown that up to 20% of CI-deficient patients carry mtDNA point mutations. These are recurrent mutations in nonrelated patients.

Leber's hereditary optic neuropathy (LHON) is also caused by primary mutations in mtDNA-encoded CI subunits (G11778A, T14484C, G15257A). Other mtDNA mutations – called secondary mutations – can act autonomously or in association [13].

NARP and several related syndromes of sensory neuropathy, seizures and mental retardation are due to a recurrent amino acid change in a subunit of CV, the *ATPase 6* gene (T8993G) [14].

By contrast, mutations in mt CIV subunits are seldom found in cytochrome-c oxidase deficiency. Only rare and private mutations in the three mitochondrial CIV subunits have been reported (COXI, COXII, COXIII). Similarly, a mitochondrial CIII mutation is a relatively rare cause of respiratory enzyme dysfunction [13].

Most of these mutations are maternally inherited, heteroplasmic and associated with strikingly various clinical phenotypes. Maternal relatives of patients are generally healthy as long as they have no more than 85% mutant mtDNA. Once the percentage of mutant mtDNA rises beyond this level there are increasingly serious clinical consequences, highlighting the sharp threshold in protein-synthesis mutations.

15.3.2 Large-scale mtDNA Rearrangements

Large-scale mtDNA deletions are usually sporadic, heteroplasmic and single. They frequently occur between directly repeated sequences, suggesting that they are caused by de novo rearrangements during oogenesis or early development. Within this group approximately 30% of patients have a common deletion (4977 bp) flanked by 13-bp direct repeats found in skeletal muscle (occasionally white blood cells) of 30% of patients harbouring a unique mtDNA deletion. They are the major cause of KSS [15], Pearson syndrome [3], PEO [16] and syndromic diabetes with deafness and multiorgan involvement.

It should be borne in mind that quantitative (depletion) and qualitative mtDNA anomalies (multiple deletion) may also result from mutations in nuclear genes involved in mtDNA maintenance (see below).

15.3.3 Nuclear DNA Mutations

Nuclear gene mutations probably underlie the vast majority of deficiencies, as mtDNA deletions and mutations account for no more than 15-20% of cases in paediatric patients. Yet, apart from those for CI deficiency, most of those nuclear genes remain unknown.

▪ Mutations in the Catalytic Subunits of the Respiratory Chain

About 40% of CI deficiencies are caused by mutations in nuclear genes encoding catalytic subunits of CI [17]. By contrast, mutations in CII are seldom found in CII deficiency (subunit A of succinate dehydrogenase) [18]. Nonetheless, mutations in subunits B-D have been reported in hereditary paraganglioma and phaeochromocytoma [19]. This suggests that those genes may be involved

in carcinogenesis. Similarly, mutations in only two genes encoding CIII (*UQCRB* and *UQCR)* [20, 21] and two genes encoding CIV have been reported (*COX4I2* and *COX6B1)* [22, 23]. No mutation in a nuclear subunit of complex V has hitherto been described.

■ Genes Involved in RC Assembly and Stability

The deficiency of a respiratory chain enzyme may also result from the impaired assembly of the corresponding complex. Indeed, an increasing number of mutations in assembly proteins are reported in respiratory chain deficiency. Mutations in assembly proteins have been reported in CI deficiency (*NDUFAF1* [24], *C6orf66* [25], *C8orf38* [25], *C20orf7* [24] and *ACAD9* [26-28]). Similarly, *SDHAF1* mutations reduce the amounts of CII and cause isolated CII deficiency [29].

Mutations in *BCS1L,* an important factor for assembly of the iron-sulfur protein subunit of CIII, have been identified in CIII deficiency with (1) tubulopathy and hepatic failure [30], (2) GRACILE syndrome [5] and (3) Björnstad syndrome [6].

Mutations in *SURF1,* which is required for haem insertion into CIV, is a major cause of Leigh syndrome with cytochrome-c oxidase deficiency [31]. Similarly, mutations in *COX10*, which encodes a haem A: farnesyltransferase (catalysing the conversion of protohaem to haem A prosthetic group) and *COX15* (hydroxylation of haem O to form haem A) caused cytochrome-c oxidase deficiency with cardiomyopathy or Leigh syndrome and tubulopathy with leukodystrophy, respectively [32, 33].

Mutations in the *SCO1* and *SCO2* genes, which are involved in mitochondrial copper maturation and synthesis of subunit II of CIV, caused cytochrome-c oxidase deficiency with hepatopathy and encephalo-cardiomyopathy, respectively [34, 35].

Mutations in *ATP12* and *TMEM70*, two transmembrane mitochondrial proteins involved in the assembly of complex V, caused CV deficiency, with dysmorphic features, neurological involvement, methylglutaconic aciduria and neonatal encephalocardiomyopathy, respectively [36, 37].

The X-linked AIFM1 gene encodes the apoptosis-inducing factor (AIF). Mutations of *AIFM1* gene have been reported in two male patients with progressive mitochondrial encephalomyopathy associated with CIII and CIV deficiency. Increased apoptosis was observed in muscle of these patients. This report suggests that AIF is involved in respiratory chain integrity and mtDNA maintenance [38].

■ Genes Involved in Coenzyme Q Synthesis

Electron transfer along the respiratory chain also depends on a quinone pool synthesised in the mitochondria. Ubiquinone deficiencies have been reported in several patients, but the primary nature of these deficiencies was proven in few patients. The two main clinical presentations are the ataxic and the myopathic forms. The ataxic form is characterised by ataxia and cerebellar atrophy, and patients present mutations in the CABC1/ADCK3 gene [39, 40]. The myopathic patients have exercise intolerance, fatigue, proximal myopathy and high serum CK. They have a CoQ_{10} deficiency in muscle and also multiple acyl-CoA deficiency, leading to the identification of ETFDH mutations and suggesting that ETFDH deficiency leads to a secondary CoQ_{10} deficiency [41]. Deficiencies in several other enzymes or proteins involved in this biosynthesis pathway (*PDSS1*, *PDSS2*, *COQ2*, *COQ9*) are reportedly associated with various clinical presentations [42-44]. Unfortunately, the small number of patients or families hampers to establish any genotype phenotype correlation.

■ Genes Involved in mtDNA Stability

Defects in mtDNA replication or dNTP supply can alter mtDNA structure and copy number, and cause multiple mtDNA deletion and mtDNA depletion respectively. This results in a combined deficiency of all mitochondrially-encoded respiratory chain enzymes (CII is normal)

Multiple mtDNA deletions (restricted to muscle tissue) are observed in autosomal dominant PEO and result from mutations in any of the following genes: *POLG1* (mtDNA polymerase γ) [45], *POLG2* [46], *ANT1* (mitochondrial ADP/ATP translocator) [47], *PEO1* (Twinkle helicase) [48] and *OPA1*, a dynamin-related GTPase involved in mitochondrial fusion [49]. In rare cases, the disease is autosomal recessive.

Mitochondrial DNA depletion syndrome (MDS) is the consequence of an abnormal mtDNA replication, particularly due to impaired dNTP supply and replication mechanisms. It is usually tissue-specific, with residual mtDNA levels below 10% of normal values. Hepatocerebral forms of MDS and/or Alpers' syndrome result from mutations in either of two replication factors, *POLG* and *PEO1* encoding the Twinkle helicase [50, 51]. They may also result from mutations in the *DGUOK* gene [52], which encodes the mitochondrial deoxyguanosine kinase involved in the salvage pathway of dNTPs for mtDNA synthesis (► Chapter 36), or the *MPV17* gene, which encodes a protein of unknown function [53]. The *TK2* gene encodes a thymidine kinase, which is also involved in mitochondrial dNTP salvage (► Chapter 36). Mutations of this gene are associated with various clinical presentations, namely severe infantile myopathy, motor neuron disease resembling spinal muscular atrophy, epileptic encephalopathy and cardiomyopathy [54].

Normal mtDNA synthesis also requires a balanced cytosolic dNTP pool. Indeed, mutations of *RRM2B* encoding a small subunit of the cytosolic ribonucleotide reductase (the enzyme that catalyses dNDP synthesis from NDP) cause a severe muscle MDS [55]. Similarly, mutations in either of the two succinyl-CoA synthase subunits (*SUCLA2*, *SUCLG1*) cause mild MDS [56, 57]. Both mutations lead to methylmalonic aciduria, which has sound predictive value in MDS though its pathogenesis is poorly understood. Similarly, mutations of the thymidine phosphorylase (TP) gene responsible for MNGIE affect the mitochondrial dNTP pool and cause mtDNA deletions and depletion [58].

- **Genes Involved in Translation of mtDNA-encoded Proteins**

Mitochondria have a specific nuclearly encoded translation machinery for mDNA-encoded proteins. Mutations in several aminoacyl-tRNA synthetases, ribosomal proteins and translation factors have been reported in patients with multiple respiratory chain enzyme deficiency and various clinical presentations, namely *PUS1* (myopathy and sideroblastic anaemia) [8], *DARS2* (leukoencephalopathy) [59], *RARS2* (pontocerebellar hypoplasia) [60], *TSFM* (encephalomyopathy or hypertrophic cardiomyopathy) [61], *GFM1* (hepatoencephalopathy) [62, 63], *EFTu* (infantile encephalopathy) [63], *MRPS16* (fatal neonatal lactic acidosis) [64], *MRPS22* (multivisceral involvement) [65] and *TRMU* (hepatic failure) [66]. Finally, mutations in LRPPRC and TACO1 altered translation of cytochrome-c oxidase subunits, and caused French-Canadian type [67] and late-onset [68] Leigh syndrome, respectively. The LRPPRC protein is thought to be involved in the translation or stability of the mRNA of subunits I and III of cytochrome-c oxidase. TACO1 is a translational activator of the mtDNA COX1 subunit.

- **Genes Involved in Mitochondrial Fusion-Fission**

Besides 'classic' mutations involving the catalytic subunits and the respiratory chain assembly, there are many newly described mutations that affect mitochondrial fusion and fission. OPA1 encodes a dynamin-related GTPase, and mutations of this gene were first reported in isolated autosomal dominant optic atrophy, but also in patients with optic atrophy associated with PEO, ataxia, deafness, sensorimotor neuropathy and multiple mtDNA deletions [49]. Recently OPA1 mutations were found in patients with, myalgia, ptosis, hearing loss, gastrointestinal dysmotility but no optic atrophy or multiple mtDNA deletion [69]. MFN2 encodes mitofusin 2, a mitochondrial membrane protein that participates in mitochondrial fusion and contributes to the maintenance and operation of the mitochondrial network. MFN2 mutations cause Charcot-Marie-Tooth disease type 2A2 and hereditary motor and sensory neuropathy VI [70, 71]. Finally, a mutation in the dynamin like protein 1 (DLP1) required for peroxysomal and mitochondrial fission presents with hyperlactacidaemia and VLCFA accumulation [71].

15.3.4 Genetic Counselling and Prenatal Diagnosis

The identification of certain clinical phenotypes, listed above, allows some predictive value with respect to their inheritance. Moreover, it should be borne in mind that, in cases of maternal inheritance of a mtDNA mutation, risk is absent for the progeny of an affected male but is high for that of a carrier female. In this case, determination of the proportion of mutant mtDNA on chorionic villi or amniotic cells is a rational approach. Nevertheless, its predictive value remains uncertain, owing to incomplete knowledge of the tissue distribution of abnormal mtDNA, its change during development and its quantitative relationship to disease severity.

In the absence of detectable mtDNA mutations, measurement of the activities of respiratory enzymes in cultured amniocytes or choriocytes provides the only possibility of prenatal diagnosis when no mutations have been identified. Yet, the ongoing identification of disease-causing nuclear genes already helps in delivering accurate prenatal diagnoses of respiratory chain deficiencies in a growing fraction of cases (particularly CI deficiency and MDS).

15.4 Diagnostic Tests

15.4.1 Screening Tests

Screening tests include the determination of lactate, pyruvate and ketone bodies, and their molar ratios in plasma as indices of oxidation/reduction status in cytoplasm and mitochondria, respectively (Table 15.2). Determinations should be made before and 1 h after meals throughout the day. Blood glucose and nonesterified fatty acids should be simultaneously monitored (► Chapter 3). The observation of a persistent hyperlactataemia (>2.5 mM) with elevated L/P and ketone body molar ratios (particularly in the postabsorptive period) is highly suggestive of a respiratory chain deficiency. In addition, investigation of the redox status in plasma can help discriminate between the different causes of congenital lactic acidosis based on L/P and ketone body molar ratios in vivo (► Chapters 1, 12). However,

Table 15.2. Screening of the respiratory chain

Standard screening tests (at least four determinations per day in fasted and 1-h-fed individuals)
Plasma lactate
Lactate/pyruvate molar ratio: redox status in cytoplasm
Ketonaemia (paradoxical elevation in fed individuals)
β-Hydroxy butyrate/acetoacetate molar ratio: redox status in the mitochondria
Blood glucose and free fatty acids
Urinary organic acids (GC-MS): lactate, ketone bodies, citric acid cycle intermediates
Provocative tests (when standard tests are inconclusive)
Glucose test (2 g/kg orally) in fasted individuals, with determination of blood glucose, lactate, pyruvate, ketone bodies and their molar ratios just before glucose administration, and then every 30 min for 3-4 h (► Chapter 4)
Lactate/pyruvate molar ratios in the CSF (only when no elevation of plasma lactate is observed)
Redox status in plasma following exercise
Screening for multiple organ involvement
Liver: Hepatocellular dysfunction
Kidney: Proximal tubulopathy, distal tubulopathy, proteinuria, renal failure
Heart: Hypertrophic cardiomyopathy, heart block (ultrasound, ECG)
Muscle: Myopathic features (CK, ALAT, ASAT, histological anomalies, RRF)
Brain: Leukodystrophy, poliodystrophy, hypodensity of the cerebrum, cerebellum and brain stem, multifocal areas of hyperintense signal (MELAS), bilateral symmetrical lesions of the basal ganglia and brain stem (Leigh) (EEG, brain MRI, NMR spectroscopy)
Peripheral nerve: Distal sensory loss, hypo- or areflexia, distal muscle wasting (usually subclinical), reduced motor nerve conduction velocity (NCV) and denervation features (NCV, EMG, peripheral nerve biopsy showing axonal degeneration and myelinated fibre loss)
Pancreas: Exocrine pancreatic dysfunction
Gut: Villous atrophy
Endocrine: Hypoglycaemia, hypocalcaemia, hypoparathyroidism, growth hormone deficiency (stimulation tests)
Bone marrow: Anaemia, neutropenia, thrombopenia, pancytopenia, vacuolisation of marrow precursors
Eye: PEO, ptosis, optic atrophy, retinal degeneration (fundus, ERG, visually evoked potentials)
Ear: Sensorineural deafness (auditory evoked potentials, brain stem-evoked response)
Skin: Trichothiodystrophy, mottled pigmentation of photo exposed areas

ALAT, alanine aminotransferase; *ASAT*, aspartate aminotransferase; *CK*, creatine kinase; *CSF*, cerebrospinal fluid; *ECG*, electrocardiogram; *EEG*, electroencephalogram; *EMG*, electromyogram; *ERG*, electroretinogram; *GC-MS*, gas chromatography-mass spectrometry; *MELAS*, mitochondrial encephalopathy with lactic acidosis and stroke-like episodes; *NCV*, nerve conduction velocity; *NMR*, nuclear magnetic resonance; *PEO*, progressive external ophthalmoplegia; *RRF*, ragged red fibre

these diagnostic tests may fail to detect any disturbance of the redox status in plasma. Pitfalls of metabolic screening are the following:

- Hyperlactataemia may be latent in basal conditions and may be revealed only by a glucose loading test (2 g/kg orally) or by determination of the redox status in the CSF. The measurement of CSF lactate and L/P ratio is useless when the redox status in plasma is altered.
- Proximal tubulopathy may lower blood lactate and increase urinary lactate. In this case, gas chromatography mass spectrometry can detect urinary lactate and citric acid cycle intermediates.
- Diabetes mellitus may hamper the entry of pyruvate into the citric acid cycle.
- Tissue-specific isoforms may be selectively impaired, barely altering the redox status in plasma (this may be particularly true for hypertrophic cardiomyopathy).
- The defect may be generalised but partial; the more those tissues with higher dependencies on oxidative metabolism suffer (such as brain and muscle), the more the oxidation/reduction status in plasma is impaired.
- The defect may be confined to complex II, barely altering (in principle) the redox status in plasma.

When diagnostic tests are negative, the diagnosis of a respiratory chain deficiency may be missed, especially when only the presenting symptom is present. By contrast, the diagnosis is easier to consider when seemingly unrelated symptoms are observed. For this reason, the investigation of patients at risk (whatever the presenting symptom) includes the systematic screening of all target organs, as multiple organ involvement is an important clue to the diagnosis (Table 15.2).

15.4.2 Enzyme Assays

The observation of an abnormal redox status in plasma and/or evidence of multiple organ involvement prompts further enzyme investigations. These investigations include two entirely distinct diagnostic procedures, which provide independent clues to respiratory chain deficiencies: polarographic studies and spectrophotometric studies.

Polarographic studies consist in the measurement of oxygen consumption by mitochondria-enriched fractions in a Clark electrode in the presence of various oxidative substrates (malate with pyruvate, malate with glutamate, succinate, palmitate, etc.) [72]. In the case of CI defi-

ciency, polarographic studies show impaired respiration with NADH-producing substrates, whilst respiration and phosphorylation are normal with FADH-producing substrates (succinate). The opposite is observed in the case of CII deficiency, whereas a block at the level of CIII or CIV impairs oxidation of both NADH- and FADH-producing substrates. In CV deficiency, respiration is impaired with various substrates, but adding the uncoupling agent 2,4-dinitrophenol or calcium ions returns the respiratory rate to normal, suggesting that the limiting step involves phosphorylation rather than the respiratory chain.

It is worth remembering that polarographic studies detect not only disorders of oxidative phosphorylation but also PDH deficiencies, citric acid cycle enzyme deficiencies and genetic defects of carriers, shuttles and substrates (including cytochrome c, cations, and adenylate), as these conditions also impair the production of reducing equivalents in the mitochondrion. In these cases, however, respiratory enzyme activities are expected to be normal.

While previous techniques required gram-sized amounts of muscle tissue, the scaled-down procedures available now allow the rapid recovery of mitochondria-enriched fractions from small skeletal muscle biopsies (100-200 mg, obtained under local anaesthetic), thus making polarography feasible in infants and children. Polarographic studies on intact circulating lymphocytes (isolated from 10 ml of blood on a Percoll cushion) or detergent-permeabilised cultured cells (lymphoblastoid cell lines, skin fibroblasts) are also feasible and represent a less invasive and easily reproducible diagnostic test. The only limitation of these techniques is the absolute requirement for fresh material: no polarographic studies are possible on frozen material.

Spectrophotometric studies consist of the measurement of respiratory enzyme activities separately or in groups, using specific electron acceptors and donors. They do not require the isolation of mitochondrial fractions and can be carried out on tissue homogenates. For this reason, the amount of material required for enzyme assays is very small and can easily be obtained by needle biopsies of liver and kidney, and even by endomyocardial biopsies. Similarly, a 25-ml flask of cultured skin fibroblasts or a lymphocyte pellet derived from a 10-ml blood sample are sufficient for extensive spectrophotometric studies. Samples should be frozen immediately and kept dry in liquid nitrogen (or at -80°C).

Particular attention should be given to apparently paradoxical cases in which respiratory enzyme activities are separately normal but deficient when tested in groups (CI-III, CII-III, CIII-V), as these are possible cases of coenzyme Q_{10} (CoQ_{10}) deficiency, a potentially treatable condition due to an inborn error of quinone synthesis. CoQ_{10} plays a pivotal role in the mitochondrial respiratory chain. It distributes the electrons between the various dehydrogenases and the cytochrome segments of the respiratory chain. It is in large excess relative to any other component of the respiratory chain and forms a kinetically compartmentalised pool, the redox status of which tightly regulates the activity of the dehydrogenases.

Since conclusive evidence of respiratory chain deficiency is given by enzyme assays, the question of which tissue should be investigated deserves particular attention. In principle, the relevant tissue is the one that clinically expresses the disease. When the skeletal muscle expresses the disease, the appropriate working material is a microbiopsy of the deltoid. When the haematopoietic system expresses the disease (i.e. Pearson syndrome), tests should be carried out on circulating lymphocytes, polymorphonuclear cells or bone marrow. However, when the disease is predominantly expressed in the liver or heart, gaining access to the target organ is far less simple. Yet, a needle biopsy of the liver or an endomyocardial biopsy is usually feasible. If not, or when the disease is mainly expressed in a barely accessible organ (brain, retina, endocrine system, smooth muscle), peripheral tissues (including skeletal muscle, cultured skin fibroblasts and circulating lymphocytes) should be extensively tested. Whichever the expressing organ, it is essential to take skin biopsies from such patients (even post mortem) for subsequent investigations on cultured fibroblasts.

It should be borne in mind, however, that in vitro investigation of oxidative phosphorylation remains difficult regardless of the tissue tested. Several pitfalls should be considered:

- A normal respiratory enzyme activity does not preclude mitochondrial dysfunction even when the tissue tested clinically expresses the disease. One might be dealing with a kinetic mutant, tissue heterogeneity, or cellular mosaicism (heteroplasmy; see above). In this case, one should carry out extensive molecular genetics analyses, test other tissues and (possibly) repeat investigations later.
- Apart from overt misdiagnosis (i.e. confusion of Pearson syndrome with Schwachman syndrome) and false respiratory enzyme deficiencies (particularly common in nonexpert centres), we are now aware of true secondary respiratory enzyme deficiency in: (1) other inborn errors of metabolism, namely propionic acidaemia, TCA cycle disorders (fumarase deficiency), fatty acid oxidation disorders (long chain and 3-hydroxy long-chain acyl-CoA dehydrogenase deficiency) and mevalonate kinase deficiency); (2) primary central nervous system (CNS) disorders,

particularly Friedreich ataxia, where iron load causes a free radical-mediated iron sulfur cluster injury in the respiratory chain; (3) chromosomal microdeletions, unbalancing the stoichiometry of the respiratory chain (i.e., 1p36 deletion).

- The scattering of control values occasionally hampers the recognition of enzyme deficiencies, as normal values frequently overlap those found in the patients. It is helpful to express results as ratios, especially as the normal functioning of the respiratory chain requires a constant ratio of enzyme activities. Under these conditions, patients whose absolute activities are in the low normal range can be unambiguously diagnosed as enzyme deficient, although this expression of results may fail to recognise generalised defects of oxidative phosphorylation.
- The phenotypic expression of respiratory enzyme deficiencies in cultured cells is unstable, and activities return to normal values when cells are grown in a standard medium. The addition of uridine (200 mM) to the culture medium avoids counterselection of respiratory enzyme-deficient cells and allows them to grow normally, thereby stabilising the mutant phenotype. (Uridine, which is required for nucleic acid synthesis, is probably limited by the secondary deficiency of the respiratory chain-dependent dihydroorotate dehydrogenase activity).
- Discrepancies between control values may indicate faulty experimental conditions. Activities dependent on a single substrate should be consistent when tested under non-rate-limiting conditions. For example, normal succinate cytochrome c reductase activity should be twice as high as normal succinate quinone dichlorophenolindophenol (DCPIP) reductase activity (because one electron is required to reduce cytochrome c, while two are required to reduce DCPIP).
- Incorrect freezing may result in a rapid loss of quinone-dependent activities, probably through peroxidation of membrane lipids. Tissue samples fixed for morphological studies are inadequate for subsequent respiratory enzyme assays.

15.4.3 Histopathological Studies

The histological hallmark of mitochondrial myopathy is the presence of ragged red fibres (RRFs), which are demonstrated using the modified Gomori trichrome stain and contain peripheral and intermyofibrillar accumulations of abnormal mitochondria. Although the diagnostic importance of pathological studies is undisputed, the absence of RRFs does not rule out the diagnosis of mitochondrial disorder. Various histochemical stains for oxidative enzymes are used to analyse the distribution of mitochondria in the individual fibres and to evaluate the presence or absence of the enzymatic activities. Histochemical staining permits an estimation of the severity and heterogeneity of enzyme deficiency in the same muscle section. Myofibrillar integrity and the predominant fibre type and distribution can be evaluated with the myofibrillar adenosine triphosphatase stain. Studies using polyclonal and monoclonal antibodies directed against CIV subunits are carried out in specialised centres. For analysis, the muscle specimen taken under local anaesthetic must be frozen immediately in liquid-nitrogen-cooled isopentane.

15.4.4 Magnetic Resonance Imaging (MRI) and Spectroscopy of Muscle and Brain

Brain imaging helps to orient genetic studies in respiratory chain deficiency. Hence, a common pattern of brain MRI imaging, with abnormal signal intensities in brain stem and subtentorial nuclei with lactate peak, has been identified in CI deficiency [73]. All patients showed bilateral brain stem lesions, and 75% showed anomalies of the putamen. Brain MRI should be regarded as an important diagnostic clue in respiratory chain enzyme deficiency.

Magnetic resonance spectroscopy (MRS) also allows the study of muscle and brain energy metabolism in vivo. Lactate, inorganic phosphate (Pi), phosphocreatine (PCr) and intracellular pH may be measured. The Pi/PCr ratio is the most useful parameter and may be monitored at rest, during exercise, and during recovery. An increased ratio is found in most patients, and MRS is becoming a useful tool in the diagnosis of mitochondrial diseases and in the monitoring of therapeutic trials. However, the observed anomalies are not specific to respiratory enzyme deficiencies, and no correlation between MRS findings and the site of the respiratory enzyme defect can be found.

15.4.5 Molecular Genetic Tests

In the absence of strict genotype/phenotype correlations, and remembering that the number of known and unknown disease genes is extremely high, the strategy of molecular genetic tests in respiratory chain defects is difficult to decide and the question of which molecular investigations should be performed first remains disputed. Briefly, the genetic tests are oriented by the clinical presentation, either as the unique criteria or in associa-

tion with other criteria, namely (1) the metabolic workup, (2) the type of enzyme deficiency and (3) the pattern of brain imaging.

Hence, recognition of a previously reported syndrome frequently helps in orienting the molecular tests, regardless of the other criteria. This is particularly true for MELAS, MERRF, LHON, NARP, KSS, PEO, MNGIE, Pearson syndrome and the diabetes mellitus-deafness association.

In patients with clinical and brain MRI evidence of Leigh syndrome, the type of enzyme deficiency helps to orient the molecular tests: *SURF1* in CIV deficiency, *SDHA* in CII deficiency, mtDNA-encoded subunits in CI deficiency. For the fast detection of mtDNA mutations, the use of Surveyor nuclease, a mismatch-specific endonuclease derived from celery, is helpful. Amplified mtDNA is denatured and then rehybridised to generate mismatches in heteroduplexed DNA, which is then treated and cleaved by the nuclease.

The observation of a multiple respiratory enzyme chain deficiency prompts the search for (1) a mtDNA deletion by long-range PCR, (2) a mtDNA depletion by quantitative PCR or (3) a defect in mitochondrial translation by Western blot analysis or Blue Native polyacrylamide gel analysis of mitochondrial proteins. In this case, a combination of additional criteria helps in orienting the genetic tests.

Hence, MDS in neonatal hepatic failure should prompt testing of the *DGUOK* and *POLG1* genes. Brain MRI evidence of bi-putaminal lesions with methylmalonic aciduria should prompt testing of *SUCLA2* and *SUCLG1*. MRI evidence of severe cortico-pontocerebellar atrophy should prompt a search for defects in mitochondrial translation (*RARS2*).

When this first screening is negative, the number of possible disease genes is so high that only future high-throughput screening/resequencing procedures will help in diagnosis of the disease-causing mutation.

15.5 Treatment and Prognosis

No satisfactory therapy is presently available for respiratory chain deficiency. Treatment remains largely symptomatic and does not significantly alter the course of the disease. It includes symptomatic treatments, supplementation with cofactors, prevention of oxygen radical damage to mitochondrial membranes, dietary recommendations and avoidance of drugs and procedures known to have a detrimental effect.

It is advisable to avoid sodium valproate and barbiturates, which inhibit the respiratory chain and have occasionally been shown to precipitate hepatic failure in respiratory enzyme-deficient children. Tetracyclines and chloramphenicol should also be avoided, as they inhibit mitochondrial protein synthesis. Owing to the increasing number of tissues affected in the course of the disease, organ transplantations are exceptional (bone marrow, liver, heart).

Symptomatic treatments include: slow infusion of sodium bicarbonate during acute exacerbation of lactic acidosis, pancreatic extract administration in cases of exocrine pancreatic dysfunction and repeated transfusions in cases of anaemia or thrombocytopenia. Recently, administration of L-arginine, a nitric oxide precursor, has been shown to significantly decrease the frequency and severity of stroke-like episodes in MELAS.

One recently identified condition, inborn errors of coenzyme Q_{10} (CoQ_{10}) synthesis, deserves particular attention as, when recognised, this condition should be treatable by large doses of oral quinone (Ubidecarenone). In the five hitherto recognised clinical presentations, the respiratory enzyme activities are individually normal but are deficient when tested as a group, as CoQ_{10} acts as an electron shuttle between complexes in the respiratory chain. Giving oral quinones to CoQ_{10}-deficient patients restores the electron flow (5 mg/kg/day) [74]. Yet, apart from this rare situation, neither CoQ_{10} nor its analogues (Idebenone) can restore electron flow in the case of respiratory chain deficiency. Oral quinones are not only useless, but even possibly harmful in respiratory chain deficiency. Indeed, because quinones can divert electrons from the respiratory chain, they may become pro-oxidant and possibly deleterious if reduced quinones are not reoxidised by a normally functioning respiratory chain. The low uptake of oral quinones by CoQ_{10}-sufficient cells probably limits their deleterious effect when they are given to respiratory enzyme-deficient patients. By contrast, in Friedreich ataxia, where iron overload causes a free-radical-induced iron sulfur cluster injury to an otherwise normal respiratory chain, idebenone (10 mg/kg/day) reoxidised on the respiratory chain has been shown to efficiently counteract the life-threatening hypertrophic cardiomyopathy [75]. Treatment with riboflavin (100 mg/day) has been associated with improvement in a few patients with complex I deficiency myopathy. Carnitine is suggested in patients with secondary carnitine deficiency. Dichloroacetate or 2-chloropropionate administration has been proposed to stimulate pyruvate dehydrogenase (PDH) activity and has occasionally reduced the level of lactic acid, but detrimental effects of dichloroacetate have recently been reported.

The dietary recommendation are a high-lipid, low-carbohydrate diet in patients with complex I deficiency.

Indeed, a high-glucose diet is a metabolic challenge for patients with impaired oxidative phosphorylation, especially as glucose oxidation is largely aerobic in the liver. Based on our experience, we suggest avoiding a hypercaloric diet and parenteral nutrition and recommend a low-carbohydrate diet in addition to the symptomatic treatment. Succinate (6 g/day), succinate-producing amino acids (isoleucine, methionine, threonine and valine) or propionyl carnitine have occasionally been given to patients with complex I deficiency, as these substrates enter the respiratory chain via complex II.

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Creatine Deficiency Syndromes

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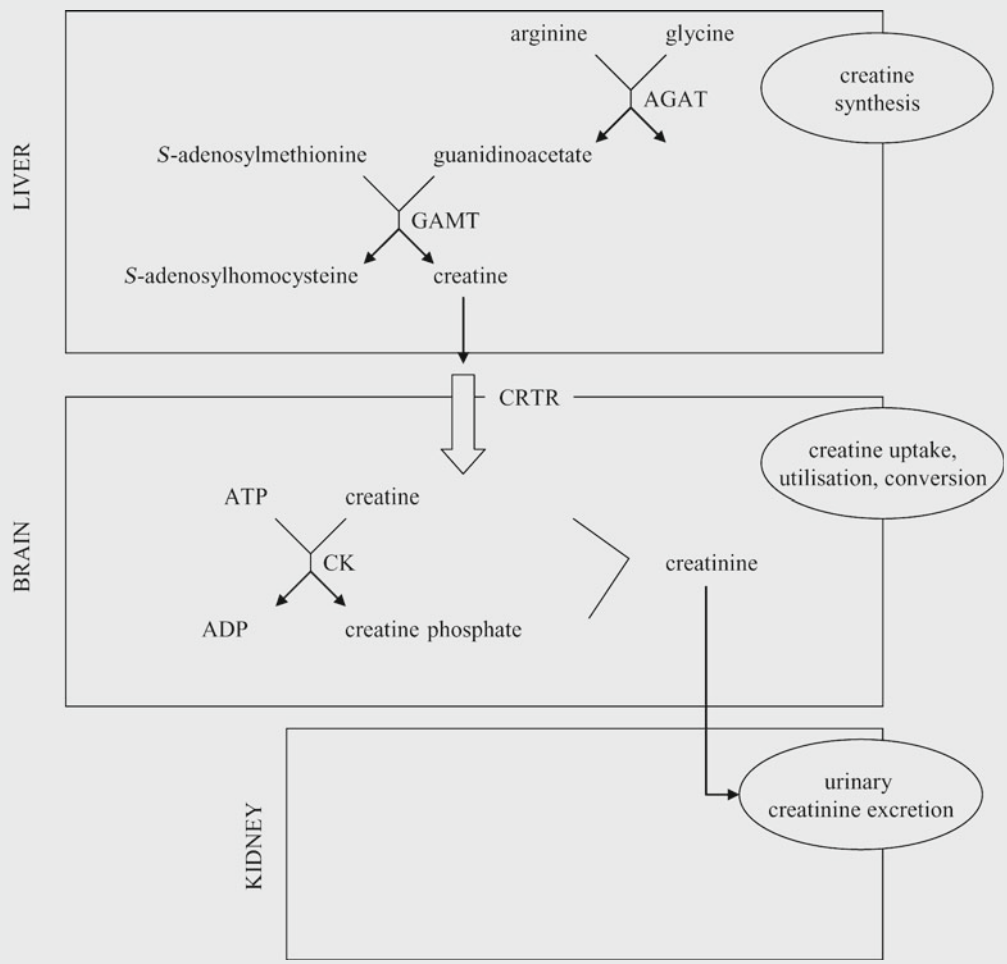
Creatine Synthesis and Transport

Creatine is synthesised by two enzymatic reactions: (1) transfer of the amidino group from arginine to glycine, yielding guanidinoacetate and catalysed by L-arginine:glycine amidinotransferase (AGAT); (2) methylation of the amidino group in the guanidinoacetate molecule by *S*-adenosyl-L-methionine:*N*-guanidinoacetate methyltransferase (GAMT) (◘ Fig. 16.1). Creatine synthesis occurs primarily in the kidney and pancreas, which have high AGAT activity, and in liver, which has high GAMT activity. From these organs of synthesis, creatine is transported via the bloodstream to the organs of utilisation (mainly muscle and brain), where both the endogenous creatine and that derived from dietary sources are taken up by a sodium- and chloride-dependent creatine transporter (CRTR, SLC6A8). Some of the intracellular creatine is reversibly converted into the high-energy compound creatine phosphate by the action of creatine kinase (CK). Three cytosolic isoforms, brain type (BB-CK), muscle-type (MM-CK) and the MB-CK heterodimer, and two mitochondrial isoforms exist. Creatine and creatine phosphate are nonenzymatically 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 (20-25 mg/kg/24 h in children and adults, and lower in infants younger than 2 years).



◘ **Fig. 16.1.** Metabolic pathway of creatine/creatine phosphate, which mainly occurs in the organs indicated. *ADP*, adenosine diphosphate; *ATP*, adenosine triphosphate; *AGAT*, arginine:glycine amidinotransferase; *CK*, creatine kinase; *CRTR*, creatine transporter (SLC6A8); *GAMT*, guanidinoacetate methyltransferase

Creatine deficiency syndromes (CDS) are a group of inborn errors of creatine synthesis and transport and include autosomal recessive arginine:glycine amidinotransferase (AGAT) and guanidinoacetate methyltransferase (GAMT) deficiencies, and deficiency of the X-linked creatine transporter (SLC6A8). In all these disorders the common clinical hallmark is mental retardation, speech delay and epilepsy. Additional frequent manifestations include failure to thrive, growth retardation, muscular hypotonia and movement disorder (mainly extrapyramidal). The common biochemical hallmark is cerebral creatine deficiency as detected by proton magnetic resonance spectroscopy (H-MRS). Increased levels of guanidinoacetate in body fluids are pathognomonic for GAMT deficiency, whereas these levels are reduced in AGAT deficiency. Increased urinary creatine/creatinine ratio is associated with SLC6A8 deficiency. Oral supplementation of creatine leads to partial restoration of the cerebral creatine pool and improvement of clinical symptoms in GAMT and AGAT deficiency. Reduction of guanidinoacetate by additional dietary restriction of arginine (and supplementation of ornithine) appears to be of additional benefit for the long-term outcome of GAMT-deficient patients. For SLC6A8-deficient patients no effective treatment is currently available. CDS may account for a considerable fraction of children and adults with mental retardation of unknown cause, and screening for these disorders (by urinary/plasma metabolites, brain H-MRS and/or a DNA approach) should therefore be included in the investigation of this population.

Secondary changes in creatine metabolim have been described mainly in disorders affecting arginine and ornithine metabolism, such as ornithine aminotransferase (OAT) deficiency and deficiency of argininosuccinate synthase and argininosuccinate lyase.

16.1 Clinical Presentation

Common clinical hallmarks of CDS are mental retardation, speech delay and epilepsy. Mental retardation ranges from mild to severe and is characteristically associated with hyperactive behaviour and autistic features [1, 2]. Movement disorder, mainly extrapyramidal, and basal ganglia changes have been observed as additional features in GAMT deficiency.

16.1.1 Guanidinoacetate Methyltransferase Deficiency

The first patient with GAMT deficiency was described in 1994 [3, 4]. This boy was considered to be normal until 4 months of age, when he was noted to have developmental arrest, hypotonia, hyperkinetic extrapyramidal movements and head nodding. His electroencephalogram (EEG) showed slow background activity and multifocal spike slow waves. Magnetic resonance imaging (MRI) revealed bilateral abnormalities of the globus pallidus consisting of hypointensities in T_1-weighted images and as hyperintensities in T_2-weighted images.

To date, more than 50 patients are known to the authors, and many of them have been published as single or groups of cases. An overview of 27 cases showed a broad clinical spectrum from mild to severe mental retardation, occasional to drug-resistant seizures and, in the most severe cases, extrapyramidal movement disorder and pathological signal intensities in the basal ganglia [5]. Findings were widely confirmed in a more recent report of a series including 8 new patients [6]. Presentations masquerading as Leigh-like syndrome and mitochondrial disease [7] or late-onset ballistic and dystonic movement disorder [8] have been reported.

16.1.2 Arginine: Glycine Amidinotransferase Deficiency

So far patients from only three unrelated families have been identified with AGAT deficiency. The first reported family includes three siblings and their cousin. Clinical features included developmental delay/intellectual disability, speech delay, autistic behaviour, occasional seizures and brain creatine deficiency that was reversible upon creatine supplementation [9-11]. One sibling diagnosed at birth and treated with creatine within the first few weeks remained asymptomatic until the age of 18 months [12]. The second family includes a 14-month-old American girl of Chinese descent, who presented with psychomotor delay, severe language impairment, failure to thrive and autistic behaviour [13]. Recently a 21- and 14-year-old pair of siblings belonging to a Yemenite Jewish family has been reported with AGAT deficiency. Both presented with a history of developmental delay, fatigability and poor weight gain. They had moderate and mild intellectual disability (IQ 47 and 60), proximal muscle weakness, moderately elevated CK levels (500-600 U/l) and myopathic electromyography. Muscle biopsy showed tubular aggregates and decreased activities of mitochondrially encoded respiratory chain enzymes [14].

16.1.3 SLC6A8 Deficiency

The first patient with SLC6A8 deficiency was reported in 2001 [15, 16]. He had mental retardation, autistic behav-

iour and speech delay. Since then at least 78 families with a total of about 170 patients (including affected males/heterozygous females) have been diagnosed [17] (www.LOVD.nl/SLC6A8). Mental retardation, speech delay, autistic behaviour and hyperactive attention deficit are the leading clinical features [18, 19]. Additional features can include muscular hypotonia, hyperextensible joints, movement disorder, short stature, and brain atrophy, discrete facial dysmorphic features and intestinal manifestations [19-21]. Neurological and psychiatric problems can be progressive in adulthood [22]. Cardiac arrhythmia, including multiple premature ventricular contractions, has also been observed in association with SLC6A8 deficiency [23]. Epilepsy is frequently present [24], and the spectrum ranges from occasional, drug-responsive seizures [19] to frequent generalised tonic clonic seizures [25] and therapy-resistant frontal lobe epilepsy [26].

Females heterozygous for the family mutation in *SLC6A8* can have learning problems/mild mental retardation [27] The most severe phenotype has been reported in a girl with mild intellectual disability, behavioural problems and intractable epilepsy [26].

The prevalence of SLC6A8 deficiency is relatively high and may be responsible for about 2% of males with X-linked mental retardation [28-32] and for 1.4% of males with sporadic mental retardation [32].

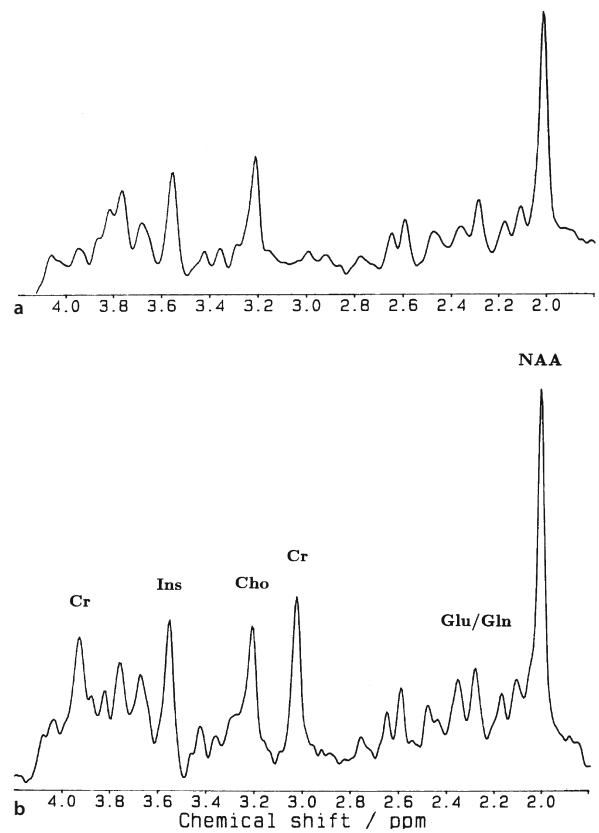
16.2 Metabolic Derangement

CDS are caused by three gene defects involved in either synthesis or transport of creatine. All three defects result in an almost complete lack of cerebral creatine (◘ Fig. 16.2). The prominent CNS involvement in all CDS patients indicates that creatine is essential for proper brain function. Apart from its role in energy storage and transmission, creatine may have an additional role as a neuromodulator [2, 33]. Although creatine plays an important metabolic role in muscle tissue, creatine levels are only slightly reduced in skeletal muscle of patients with CDS [34, 35]. Creatine has not been measured in heart muscle, but none of the reported patients had cardiomyopathy. Plasma creatine levels are largely influenced by individual nutritional facts. Thus, normal plasma creatine levels do not exclude the presence of CDS. Urinary creatine excretion is expected to be low in patients with defects in creatine synthesis, but in SLC6A8 deficiency the urinary creatine-to-creatinine ratio is high [36]. Impaired function of SLC6A8 in renal tubular cells and subsequent reduced tubular (re)uptake of creatine is the most likely reason for this. Moreover, low intracellular creatine and creatine phosphate results in reduced production of creatinine. Thus, plasma creatinine concentrations, and in particular urinary creatinine excretion, are low in patients with CDS [37].

Guanidinoacetate is the second metabolite that plays a role in CDS. In GAMT deficiency, guanidinoacetate accumulates in tissues and body fluids [2, 5]. In the CSF levels up to more than 100-fold normal are found [5]. In AGAT deficiency guanidinoacetate is low [2, 9, 11]. In SLC6A8 deficiency, guanidinoacetic acid levels are normal [36, 37].

S-Adenosylmethionine is methyldonor for creatine synthesis. Although one might speculate that S-adenosylmethionine accumulates in creatine synthesis defects, S-adenosylmethionine and S-adenosylhomocysteine levels were unremarkable in one patient with GAMT deficiency (unpublished).

Secondary (cerebral) creatine deficiencies have been observed in argininosuccinate lyase deficiency (argininosuccinic aciduria), argininosuccinate synthase deficiency



◘ **Fig. 16.2 a, b.** In vivo proton magnetic resonance spectroscopy (^{1}H MRS) of the brain of a patient with cerebral creatine deficiency due to GAMT deficiency. **a** Complete lack of creatine resonance; **b** partial normalisation of creatine spectrum after 6 months of treatment with oral creatine monohydrate

(citrullinaemia type 1) [38], and ornithine aminotransferase (OAT) deficiency (gyrate atrophy of the choroid and retina) [39]. Secondary changes in creatine metabolism seem also to occur in disorders of remethylation, such as in cobalamin C deficiency [40].

16.3 Genetics

The genes encoding for GAMT and AGAT (*GAMT* and *GATM*) are mapped on chromosome 19p13.3 and 15q15.3, respectively. Both disorders are inherited autosomal recessively, and many of the reported patients are the products of consanguineous marriages [5, 10].

The *SLC6A8* gene has been mapped to Xq28. As SLC6A8 deficiency is an X-linked disorder, males are mainly affected, while according to the X-inactivation pattern heterozygous females may have a variable clinical phenotype [27]. Moreover, it should be noted that the X-linked pattern of inheritance will not be observed in the case of a de novo mutation. Therefore, diagnostic screening of males with sporadic mental retardation (prevalence of 1.4%; CI: 0-3.30 [31] and females (prevalence data unknown) should include screening for SLC6A8 deficiency [41].

To date, in the *GAMT* and *SLC6A8* genes many different mutations have been identified, including nonsense, missense, splice error, insertion, deletion and frameshift mutations without a clear phenotype-genotype correlation [2, 5, 6, 42]. In the *GATM* gene three mutations have been found in three unrelated families; a nonsense mutation [9, 11], a splice error [12] and single nucleotide insertion [13]. There is no evidence for a hotspot region in any of these genes; however, certain mutations appear to occur more frequently. In *GAMT*, c.327G>A and c.59G>A occur in more than 50% of alleles. While c.327G>A occurs in all ethnicities [5, 6], c.59G>A has been found in patients from Southern Europe and Turkey only. This mutation is particularly prevalent in Portugal [43]; in the *SLC6A8* gene, c.319_321delCTT, and c.1221_1223delTTC) are the most frequently found [2], and at present 36 different pathogenic mutations have been reported [17] (www.LOVD.nl/SLC6A8).

16.4 Diagnostic Tests

16.4.1 MRS of Brain

Inborn errors of creatine metabolism (biosynthesis and transport) can be recognised by the marked reduction of the creatine signal in H-MRS of the brain. However, metabolite screening and molecular analysis remain necessary, to unravel the underlying defect in particular. Moreover, MRS in infants and children often requires general anaesthesia and is not generally available as a routine method. Therefore, H-MRS of the brain is not a convenient primary screening tool, even though it is increasingly becoming a part of current practice for investigating mental retardation and neurological syndromes.

16.4.2 Metabolite Screening

Analysis of urinary guanidinoacetate and the creatine-to-creatinine ratio is an important screening test for all CDS [37]. Various methods have been developed for the determination of these compounds [44]. Stable isotope gas chromatography-mass spectrometry (SID GC–MS) is a highly sensitive technique suitable for detection of low guanidinoacetate levels characteristic of AGAT deficiency and those required for analysis of this compound in the CSF. Liquid chromatography tandem mass spectrometry (LC-MS-MS) allows the rapid, simultaneous determination of urinary analytes including guanidinoacetate, creatine and creatinine [45, 46]. High guanidinoacetate levels characteristic of GAMT deficiency can be detected with all these methods.

In patients with SLC6A8 deficiency, the increase of urinary creatine excretion together with the inherently low urinary creatinine excretion results in an elevation of the urinary creatine-to-creatinine ratio, which serves as a valuable diagnostic marker in males [36, 47]. In heterozygous females this biochemical trait is not sufficiently sensitive to serve as a diagnostic marker [27]. Even in symptomatic patients the creatine-to-creatinine ratio can be within the control range [26].

Variation of these compounds during the day is not significant, indicating that a random urine sample is sufficient for the diagnosis of CDS [36]. On the other hand, false-positive values can be detected as the result of a protein-rich diet [32].

Age-dependent normal values have been established [36, 47, 48]. The urinary creatine-to-creatinine ratio decreases after the age of 3 years, while it increases during the first 3 years of life [47]. It is of note that in metabolic urine screening, an overall increased concentration of amino acids and organic acids, expressed as millimoles per mole of creatinine, may be a result of a decreased creatinine excretion and thus represent a suggestive hint for the presence of CDS [37].

Guanidinoacetate can also be measured in dried blood spots [49, 50], thus allowing newborn screening for GAMT deficiency.

16.4.3 DNA Diagnostics

Mutation analysis for the three genes (*GAMT*, *GATM* and *SLC6A8*) involved in CDS is currently used to confirm the diagnosis. Denaturing gradient gel electrophoresis (DGGE) and denaturing high-performance liquid chromatography (HPLC) methods [51, 52] were applied in the past, allowing screening of larger sample numbers. Most patients, however, have been diagnosed individually via direct gene sequencing. New technologies such as high-throughput sequencing, will allow direct gene testing as a screening tool. This might help to diagnose more patients with AGAT deficiency and SLC6A8 deficiency, for which the currently available biomarkers are not very sensitive. This is especially true for detection of heterozygous SLC6A8 females [27].

16.4.4 Functional Tests/Enzymatic Diagnostics

Functional tests and/or enzymatic diagnostics in fibroblasts and/or lymphoblasts or in expression systems facilitate confirmation of a diagnosis at a functional level, in particular if new mutations with unknown pathogenicity are detected [53-55].

16.4.5 Prenatal Diagnosis

Prenatal diagnosis and preimplantation genetic diagnosis for at-risk pregnancies require prior identification of the disease-causing mutation(s) in the family for all three creatine deficiency syndromes [1]. In addition, GAMT deficiency can be prenatally diagnosed by guanidinoacetate measurement in the amniotic fluid in pregnancies at risk for GAMT deficiency if the underlying disease-causing mutations have not been identified in the index patient [56].

16.5 Treatment and Prognosis

16.5.1 GAMT Deficiency

Oral creatine substitution has been effective in replenishing the cerebral creatine pool to approximately 70% of normal in all patients [5, 57]. Most received creatine monohydrate at 300-400 mg/kg/day in three to six divided doses. The clinical response to oral creatine supplementation alone included resolution of extrapyramidal signs and symptoms, and in most patients considerable improvement of their epilepsy [4, 5]. Additional dietary restriction of arginine helps to reduce accumulated guanidinoacetate [58], which in high concentrations is neurotoxic. Arginine is restricted to 15-25 mg/kg/day (corresponding to 0.4-0.7 g/kg/day protein intake), and supplementation with an arginine-free aminoacid mixture is necessary to provide an adequate nutritional amino acid supply [59]. Supplementation with high dosages of ornithine has the potential to reduce guanidinoacetate synthesis by competitive inhibition of AGAT activity in vitro; however, this approach did not result in reduction of guanidinoacetate in one patient [60]. A combination of arginine restriction and ornithine supplementation might be more effective. This approach has led to an impressive improvement of epileptic seizures, mental capabilities and behaviour in a severely affected adult patient [34]. In this patient, sodium benzoate was given in addition to prevent ammonia accumulation due to possible lack of arginine as the essential amino acid in the urea cycle.

Initiation of combined therapy at an early age, preferably in the neonatal period, might improve the long-term outcome. As an example, a sibling of one index patient was identified by positive mutation analysis in the neonatal period. Treatment was initiated at age 3 weeks in the presymptomatic stage of the disease. The child had a normal neurodevelopmental outcome at age 14 months compared in contrast to the older, symptomatic sibling at the same age [61]. This finding raises the question of adding GAA to the routine derivatised MS/MS newborn screen, as it is simple and adds little to the cost [62].

16.5.2 AGAT Deficiency

Oral creatine supplementation (300-400 mg/kg/day) has been effective in replenishing the cerebral creatine pool and in improving abnormal developmental scores [10-12]. Early diagnosis and treatment seem to be particularly efficient in improving outcomes. One child with global developmental delay at the age of 16 months had achieved normal developmental scores at the age of 40 months, after 23 months of treatment with creatine [13]. Another child diagnosed at age 2 years had a borderline IQ at age 8 years, whereas two children in the same family who started treatment at ages 7 and 5 years had moderate intellectual deficit at the ages of 13 and 11 years [11]. An additional patient from the same family was diagnosed prenatally, and creatine supplementation was started at 4 months. This child showed normal development in at age 18 months, in contrast to his siblings, who had already shown signs of retardation at this age [12].

16.5.3 SLC6A8 Deficiency

SLC6A8 deficiency appears not to be treatable by any of the approaches described above. Treatment of both males and females affected with SLC6A8 deficiency with creatine monohydrate has not proved successful [18]. Only one heterozygous female patient with learning disability and a mildly decreased creatine concentration on brain MRS showed mild improvement on neuropsychological testing after 18 weeks of treatment with creatine monohydrate (250-750 mg/kg/day) [16]. Supplementation with high doses of L-arginine and L-glycine, which are the primary substrates for creatine biosynthesis, combined with high doses of creatine monohydrate, are currently being investigated. The rationale behind this protocol is based on an increased cerebral uptake of both amino acids with the aim of enhancing intracerebral creatine synthesis [63]. Supplementation of L-arginine alone has resulted in developmental progress in one male patient [64]. In four male patients this treatment has failed to improve intellectual outcomes [65]. Additional supplementation of L-glycine might enhance the therapeutic effect. Combined treatment with creatine, arginine and glycine has resulted in a significant improvement of intractable seizures in a female patient with SLC6A8 deficiency [26]. In addition, alternative strategies may be developed that facilitate creatine transport into the brain (e.g. by modified transport via carrier peptides/molecules).

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IV Disorders of Amino Acid Metabolism and Transport

Hyperphenylalaninaemia

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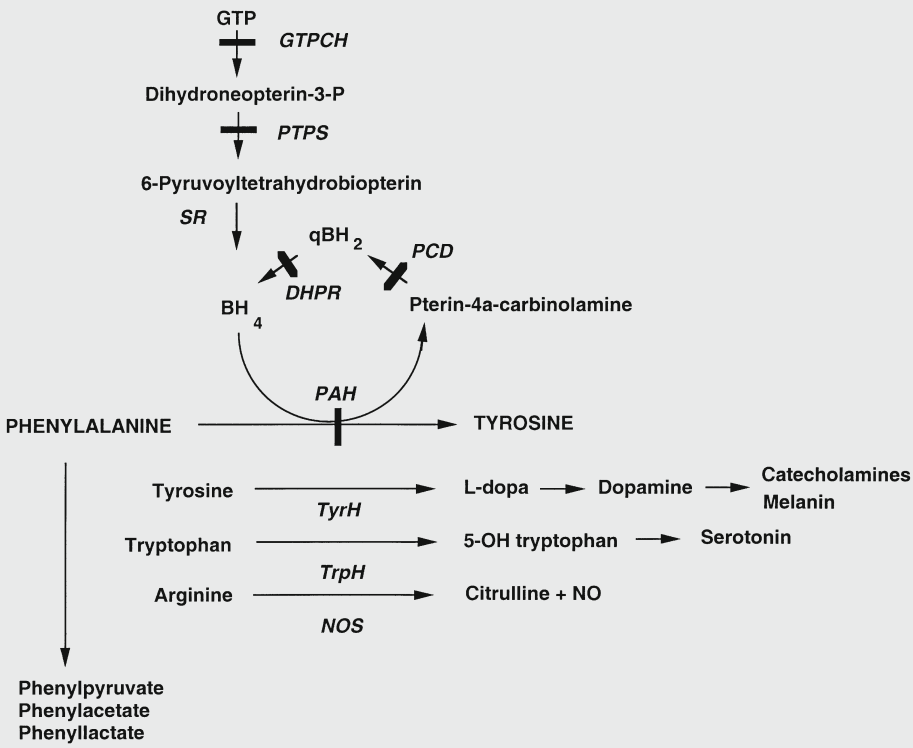
Phenylalanine Metabolism

Phenylalanine (PHE), an essential aromatic amino acid, is mainly metabolised in the liver by the PHE hydroxylase (PAH) system (◘ Fig. 17.1). 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 nitric oxide synthase.

Defects in either PAH or the production or recycling of BH_4 may result in hyperphenylalaninaemia, 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. 17.1.** The phenylalanine hydroxylation system, including the synthesis and regeneration of pterins and other pterin-requiring enzymes. *BH2,* dihydrobiopterin (quinone); *BH4,* 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 enzymes of pterin metabolism are associated with hyperphenylalaninaemia (HPA). Phenylketonuria (PKU) is caused by a severe deficiency in PAH activity and if left untreated leads to permanent central nervous system damage. Dietary restriction of phenylalanine (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. Continued dietary treatment into adulthood with PKU is generally recommended, but as yet there is insufficient data to know

whether this is necessary. 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 fetal 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 tetrahydrobiopterin and neurotransmitters.

17.1 Introduction

Defects in either phenylalanine hydroxylase (PAH) or the production or recycling of tetrahydrobiopterin (BH_4) may cause hyperphenylalaninaemia. Severe PAH deficiency, which results in a blood phenylalanine (PHE) greater than 1200 μM when individuals are on a normal protein intake, is referred to as classic phenylketonuria or just PKU. Milder defects associated with levels between 600 μM and 1200 μM are termed HPA, and those with levels less than 600 μM but above 120 μM, 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.

17.2 Phenylalanine Hydroxylase Deficiency

17.2.1 Clinical Presentation

PKU was first described by Følling in 1934 as Imbecillitas phenylpyruvica [1]. The natural history of the disease 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 severe mental retardation (IQ ≤ 50), often associated with a mousy 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 that can occur 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, purposeless movements, stereotypy, aggressiveness, anxiety and social withdrawal. The clinical phenotype correlates with PHE blood levels, reflecting the degree of PAH deficiency.

17.2.2 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 [2]. 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.

17.2.3 Genetics

PAH deficiency is an autosomal-recessively transmitted disorder. The PAH gene is located on the long arm of chromosome 12. At the time of writing 560 different mutations have been described (see http://www.pahdb.mcgill.ca). Most subjects with PAH deficiency are compound heterozygous, harbouring two different mutations. Although there is no single prevalent mutation, certain ones are more common in different ethnic populations. For example, the R408W mutation accounts for approximately 30% of alleles in Europeans with PKU, whereas in Orientals the R243Q mutation is the most prevalent, accounting for 13% of alleles. The prevalence of PAH deficiency varies between different populations (for example it is 1 in 1,000,000 in Finland and 1 in 4200 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, pretreatment PHE levels and PHE tolerance [1], with the less severe mutation in those who are compound heterozygotes defining this. However, owing to the many other factors that effect clinical phenotype, correlations between mutations and neurological, intellectual and be-

havioural outcome are weak. Mutation analysis is consequently of limited practical use in clinical management, but may be of value in determining genotypes associated with possible BH_4 responsiveness (http://www.biopku.org/BioPKU_DatabasesBIOPKU.asp).

17.2.4 Diagnostic Tests

Blood PHE is normal at birth in infants with PKU, but rises rapidly within the first days of life. In most Western nations PKU is detected by newborn population screening. There is variation between different countries and centres in the age at which screening is undertaken (day 1 to day 10), 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 DHPR in blood (► Section 17.4). Persistent hyperphenylalaninaemia may occasionally 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). In some centres the diagnosis is further characterised by DNA analysis.

PAH deficiency may be classified according to the concentration of PHE in blood 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),
- BH4-Responsive PKU/HPA (blood PHE levels decrease substantially after oral administration of BH4, thus increasing dietary PHE tolerance (► Section 17.2.5, »Treatment with BH_4«).

Although in reality there is a continuous spectrum of severity, such a classification has some use in terms of indicating the necessity for and type of treatment.

Although rarely requested, prenatal diagnosis is possible by means of PAH, DNA analysis on chorion villi biopsy (CVB) or amniocentesis where the index case has mutations identified previously.

17.2.5 Treatment and Prognosis

Principles of Treatment

Dietary Treatment

The principle of treatment in PAH deficiency is to reduce the blood PHE concentration sufficiently to prevent the neuropathological effects. 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. A PHE blood level while on a normal protein-containing diet defines the indication for treatment, with some minor differences in cut-offs: UK >400 μmol/l, Germany >600 μmol/l, and USA (>360-600 μmol/l. In all published recommendations for treatment, target blood PHE levels are age related but show substantial variation. The same is true for management practices across Europe [3]. Table 17.1 shows recommendations for UK [4] Germany [5] and the USA [6], France [7], and the Netherlands [8].

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/100g; e.g. carbohydrate, fruit and some vegetables).
- Calculated amounts of restricted natural and manufactured foods with medium PHE content (30-100 mg/100g; e.g. potato, spinach, broccoli; some kinds of 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 supplemented with vitamins, minerals and trace elements.

Intake of these three components – including the PHE-free amino acid mixture – should be distributed as evenly as possible 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 diet of patients with PKU.

PHE-free amino acid infant formulas that also contain adequate essential fatty acids, minerals and vitamins are

Table 17.1. 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
0-2 years	130-400	40-240	120-360	120-360	120-300	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		120-900	<1500	120-600

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.

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 [9]; sapropterin dihydrochloride (Kuvan®), a synthetic formulation of the active 6R-isomer of tetrahydrobiopterin is now approved in Europe for the treatment of patients with HPA and PKU who are 4 years of age or over and have been shown to be responsive to such treatment, and in the US for all ages. The most frequent definition of BH_4 responsiveness is a reduction of ≥30% in blood PHE level after a single dose of 10 mg BH_4/kg body weight, but there are also alternative suggestions [10], as the criterion of 30% depends on the initial PHE value. Studies on the PKU Pah^{enu1} mouse model, which is associated with a mild hyperphenylalaninaemia phenotype, and expression studies of mutations found in BH_4-responsive patients have shown that reduced function of PAH can be a consequence of misfolding, aggregation and accelerated degradation of the enzyme and that BH_4 may act as a chaperone, providing conformational stabilisation and augmenting the effective PAH concentration [11, 12]. Treatment with BH_4 consists of single daily doses of 5-10 mg/kg body weight, with the aim of decreasing blood PHE levels or increasing dietary PHE tolerance. As not only those patients with mild PKU, who have a higher residual PHA activity, have been shown to be responsive, genotype is not the only predictor and it has been recommended that all patients with PKU be tested for BH_4 responsiveness [13]. Both the manufacturer's prescribing information [14] and a US FDA drug review recommend that Kuvan be used in combination with a PHE-reduced diet, leaving open the question of monotherapy with BH_4 in those patients who would end up with PHE levels below the cut-off for treatment. There are no serious side effects in the short term, and from clinical trials it has been concluded that 20% of all screened patients are BH_4 responsive. However, given the limitations of the 30% criterion in determining BH_4 responsiveness and the fact that patients with classic PKU are rarely found to be responsive, it is not possible to predict the proportion of patients who might benefit significantly from long-term treatment [13, 15]. As there are no data regarding BH_4 treatment of pregnant women with PKU, diet should be the first-line treatment for pregnant women and BH_4 only used in cases of severe noncompliance [16]. Despite increased cost and regimen complexity, treatment with BH_4 will result in a substantial improvement of the quality of life in a subgroup of patients with PKU.

■ Monitoring of Treatment

The constraints of a diet that is ultimately focused at the threshold of a calculated PHE intake bears the risk of nutrient deficiency. Therefore, the treatment must be monitored by regular control of dietary intake and blood PHE levels, as well as neurological, physical, intellectual and behavioural development. Table 17.2 summarises recommendations for monitoring treatment and outcome of PKU.

■ Alternative Therapies/Experimental Trials

Although dietary treatment of PKU 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.

Table 17.2. Recommendations for monitoring treatment and outcome of PKU

Patient age	Monitoring	
	Blood PHE levels	**Clinical monitoring**[1]
0–3 years	Weekly	Every 3 months
4–6 years	Fortnightly	Every 3-6 months
7–9 years	Fortnightly	Every 6 months
10–15 years	Monthly	Every 6 months
Adolescent/ adult	Every 2-3 months	Yearly

[1] Length/height, head circumference, general status of health, neurology and psychological development. When PHE levels are within the recommended range; in general no additional routine laboratory analysis is necessary. A complete fasting profile of all amino acids, minerals, vitamins and trace elements, blood count, Ca, P metabolism, fatty acids may be indicated in individuals with poor compliance

- *Gene therapy*. Different PAH gene transfer vehicles have been tried in the PAH^{enu2} mouse. These have included nonviral vectors, recombinant adenoviral vector, recombinant retroviral vector and recombinant adeno-associated virus vector [17]. So far none of these experiments has resulted in sustained phenotypic correction, whether due to poor efficiency of gene delivery, the production of neutralising antibodies or the lack of co-factor in nonhepatic target organs. The development of a safe and more successful gene transfer vector is still required before clinical trials in humans are likely to become possible.
- *Liver transplantation* fully corrects PAH deficiency [18], 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 phenylalanine hydroxylase (PAH)-expressing cells following hepatocyte or haematopoietic stem cell transplantation [19].
- *Phenylalanine ammonia lyase*. Recombinant *Anabaena variabilis* phenylalanine ammonia lyase was able to 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 converts PHE to a harmless compound, transcinnamic acid, and is well tolerated by human subjects, with 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 in patients with PKU. Ongoing trials (phase 2) are assessing the safety and efficacy of repeated doses of rAvPAL-PEG in patients with PKU. PAL could also be an effective treatment for patients with classic PKU.
- The *large neutral amino acids* (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. Studies in the PAH^{enu2} mouse model and in patients have revealed a reduction in brain PHE levels and some positive effect on neuropsychological functions when LNAAs (apart from PHE) have been given enterally [20-22]; however, the greatest benefit may be to patients who are unable to comply with the more efficient conventional dietary treatment.

Compliance with Treatment

Compliance with treatment is most often satisfactory in infancy and childhood. However, the special 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. It has been shown that up to the age of 10 years only 40% of the sample of the German Collaborative Study of PKU have been able to keep their PHE levels in the recommended range [23] and that after the age of 10 years 50-80% of all blood PHE levels measured in a British and Australian sample were above recommendation [24].

Dietary treatment of PKU is highly demanding for patients and families and 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 problems related to a life-long diet. It is of fundamental importance that all professionals, and the families themselves, fully understand the principle 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 [3].

Outcome

The outcome for PKU is dependent upon a number of variables, which include the age at start of treatment, blood PHE levels in different age periods, duration of periods of blood PHE deficiency and individual gradient for PHE transport across the blood-brain barrier. Further unidentified co-modifiers of outcome are also likely. However, the most important single factor is the blood

PHE level in infancy and childhood. Longitudinal studies of development have shown that 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, and that 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 corresponding 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-month period of insufficient PHE intake. After the age of 10 years all studies show stable IQ performance until early adulthood irrespective of PHE levels, and a normal school career if compliance during the first 10 years has been according to treatment recommendations [25-28]. However, longitudinal studies covering middle and late adulthood are still lacking [29]. Quality of life has become an important concept in the evaluation of the outcome of treatment. Although PKU is a chronic disease with the burden of strict dietary control, early and continuously treated patients with PKU can have a normal health-related quality of life and course of life [30, 31].

■ Complications in Adulthood

■■ Neurological Abnormalities

Neuropsychological studies of reaction times demonstrate a life-long, but reversible, vulnerability of the brain to increased concurrent PHE levels [32]. A meta-analysis of neuropsychological studies has shown that adolescents and adults with PKU do differ significantly from matched control groups in tests of attention, inhibition, processing speed and motor control, with processing speed having the largest effect size [33]. However, this analysis did not include blood PHE concentrations. In a meta-analysis including concurrent blood PHE levels, effects were more pronounced in children and adolescents than in adults, with choice reaction time being particularly sensitive to PHE concentrations. These results suggest an upper threshold for PHE concentrations of 320 mol/l for children (7-12 years) and 570 mol/l for adolescents (13-18 years). In adults the negative effects remained the same between PHE concentrations of 750-1500 mol/l [34].

Nearly all patients show white matter abnormalities in brain MRI after longer periods of increased PHE levels. However, in all but one study these abnormalities have not been found to correlate with intellectual or neurological abnormalities and were reversible after 3-6 months of strict dietary treatment [35].

Patients with poor dietary control during infancy show behavioural impairments, such as hyperactivity, temper tantrums, increased anxiety and social withdrawal, most often associated with intellectual deficits. Well-treated subjects may show an increased risk of depressive symptoms and low self-esteem. However, without correlation to concurrent PHE levels, causality of this finding remains obscure, but it is hypothesised to be a consequence of living with a chronic condition rather than a biological effect of increased PHE levels [36].

A very small number of adolescent and adult patients have developed frank neurological disease, which has usually improved on returning to dietary treatment [37]. It appears that these individuals have usually had poor control in childhood. The risk to those who have been under good control in childhood and who have subsequently relaxed their diet is probably very small. In some cases neurological deterioration has been related to severe vitamin B_{12} deficiency (► below, 'Dietary Deficiencies') compounded by anaesthesia using nitrous oxide [38].

■■ Dietary Deficiencies

Vitamin B_{12} deficiency can occur in adolescents and adults who have stopped their vitamin supplements but continue to restrict their natural protein intake [39]. For patients on a strict diet there have been concerns regarding possible deficiencies in other vitamins and minerals, including selenium, zinc, iron, retinol and polyunsaturated fatty acids. However, such deficiencies are inconsistently found and it is unclear whether they are of any particular clinical significance. Low calcium, osteopenia and an increased risk of fractures have also been reported. Long-chain omega-3 polyunsaturated fatty acids (LC-PUFA), 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 has been shown to increase visual evoked potentials and motor performance in a standardised test, but the optimal type and dose of supply still needs to be determined [40].

■■ Diet for Life

Adults with PKU face a different set of challenges from children, and it is much more difficult to be proscriptive about their dietary management. Although intellectually and biochemically attractive, the concept of diet for life does pose real challenges for those who are expected to follow it. 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. Even those who wish to stay on diet often find that when they leave the parental home they lack the skills, financial resources and time required. With suitable support and education these problems can be overcome, but in any case, we know that the majority of adults are poorly compliant with dietetic advice [24]. This is likely to be because most adults with

PKU have had a period when their diet has lapsed and, in the majority of cases, they have noticed no ill effects. Given the lack of evidence for any irreversible effects of phenylalanine on the adult brain, and the inability of experts to agree on what constitutes adequate dietary control for an adult (target ranges for PHE vary by almost 1000 μmol/l across Europe) it can be difficult to persuade those 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 to ensure that they are following a nutritionally adequate diet and to monitor the long-term outcome of early-treated PKU. An evangelical approach to diet for life is likely to provide a major disincentive for these people to continue to attend the metabolic clinic. In reality, a pragmatic approach is likely to be most productive, giving adults with PKU support to follow their own choices. They can be kept informed of developments in the field in terms of new evidence and treatments, and those who choose to return to diet, for whatever reason, can be offered the training and resources they need. Because of these differences in approach, where possible adults with PKU should be cared for by metabolic physicians and dietitians with a training in adult medicine rather than by paediatricians.

▪ 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. The most severely affected will be in residential care, but many remain dependent on their parents. Many of these older people with PKU were either never treated or came off a low-protein diet an early 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 [41]. Therefore, 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, all these individuals will need alternative arrangements to be made for their long-term care. This is best done with the participation of their parents, whilst they are still able to provide help and support in choosing appropriate accommodation and settling into new surroundings. If arrangements have to be made in an emergency, because of ill health or death of a carer, the results can be disastrous.

17.3 Maternal PKU

17.3.1 Clinical Presentation

Before the introduction of newborn screening and early dietary treatment, it was unusual for women who were known to have PKU to have children of their own: in fact, as with other women with learning difficulties, positive steps were often taken to control their fertility. Early observations that some children of mothers with PKU also had a clinical phenotype of learning difficulties and behavioural problems were interpreted as some form of genetic transmission rather than an environmental problem. The first published description of the maternal PKU syndrome recognised that the teratogenicity was in fact secondary to exposure to high levels of maternal PHE in utero [42]. Initial reports recognised the occurrence of developmental delay (92%), microcephaly (73%), cardiac defects (12%), low birth weight (40%) and dysmorphic features in the infants of mothers with untreated classic PKU [43].

Although the pathogenesis of this condition is still poorly understood, much progress has been made and, as discussed below, the maternal PKU syndrome is now a preventable disease. Nonetheless, in countries where HPA is not picked up on newborn screening, affected children continue to be born.

17.3.2 Metabolic Derangement

▪ Teratogenic Effects of Phenylalanine

The Maternal PKU Collaborative Study (MPKUCS), initiated in 1984 to investigate the efficacy of dietary treatment in preventing the maternal PKU syndrome [44], also allowed a more detailed assessment of the teratogenic effects of PHE.

Widaman and Azen [45] have performed a careful analysis of the association between maternal PHE levels and developmental delay. They assessed 413 children who had developmental assessments neonatally and then at 1, 2, 4 and 7 years old. The relationship between PHE exposure (measured as the average PHE level during pregnancy) and developmental outcome was not linear. Instead, they found that for maternal PHE levels below about 360 μmol/l there was no evidence of any deleterious effect on the fetus. 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) is a much less prevalent feature of maternal PKU syndrome than developmental delay (12% vs 92%). In the MPKUCS, there were

34 cases of CHD in 235 infants born to mothers whose average PHE level was ≥900 μmol/l [46], with coarctation of the aorta and tetralogy of Fallot being the most common. There were no cases in the 131 infants from pregnancies where the mother obtained metabolic control by the 8th week of pregnancy and maintained an average PHE level of <900 μmol/l thereafter. The incidence in the control, non-PKU population was only 1%. Hence, the threshold PHE level for damage to the fetal heart seems to be considerably higher than that for fetal brain damage. The risk of CHD increases with increasing PHE exposure; 50% of mothers who had children with CHD had average PHE levels ≥1500 μmol/l. Poor metabolic control between 4 and 8 weeks of pregnancy, the time at which the heart is developing, was the strongest predictor of CHD.

For both developmental outcomes and CHD, average maternal PHE levels during pregnancy were the strongest predictor and there was no independent association with either maternal genotype or the PAH allele inherited by the affected offspring [47].

17.3.3 Treatment and Prognosis

▪ Prevention of the Maternal PKU Syndrome

Once the teratogenic effect of PHE became clear, many centres started to institute strict dietary control for women with PKU who were pregnant or, preferably, who were planning pregnancy. In the original survey of Levy and Lenke, only 6.4% of women were on a PHE-restricted diet at any stage of pregnancy and only 0.6% started such a diet prior to conception [43]. In the MPKUCS [48], 26% of women were on preconception diet, and of those entered in the UK PKU Registry [49], which was started in 1964, 30.5% commenced diet before becoming pregnant. Publications from these large studies, as well as numerous smaller samples from around the world, have clearly shown that the institution of strict metabolic control before conception is associated with normal pregnancy outcomes.

The plasma PHE targets used in maternal PKU have changed over time. Initial studies aimed to obtain levels less than 600 μmol/l. Obtaining this degree of control certainly improved outcomes and, as would be expected, reduced the incidence of CHD to background levels. The decision of some centres to aim for even lower levels was based on the fact that in infancy PHE levels of less than 360 μmol/l were required to minimise the risk of brain damage and the observation that active placental transport led to an enrichment of PHE in the fetal circulation [50]. In the UK, a target range of 100-250 μmol/l was set. With the emergence of data relating PHE exposure to developmental outcome, suggesting that below an average maternal PHE level of 360 μmol/l there is no evidence for an effect on neurodevelopment, these target ranges have been relaxed somewhat.

▪ Current Practice

Looking after pregnant women with PKU is highly intensive in terms of time and resources. As with most things in medicine, the best outcomes are obtained by the centres with the most experience [51]. In 109 pregnancies cared for in a single centre over a 30-year period, preconception diet was established in 69.5% [52]. This centre currently looks after 15-20 PKU pregnancies annually. Prospective mothers are given the opportunity to receive dietary education, with partners or families, in the dedicated metabolic kitchen. Women have PHE monitoring twice a week before conception and three times a week in pregnancy. The samples provided are analysed and the women informed of the results and any dietary changes needed on the day one of their blood spots arrives in the laboratory. Pregnant women are seen in the outpatient clinic every 6-8 weeks. To provide this level of service, and obtain the outcomes which go with it, requires the input of clinicians specifically trained in metabolic medicine, specialist dietitians, a dietetic assistant (metabolic cook) and specialised laboratory services, as well as access to excellent fetal medicine services in the inevitable cases where things do not go according to plan and neuropsychologists to monitor outcomes. These resources are only available in large, dedicated units caring for adults with inherited metabolic disease, and any woman with PKU who is either pregnant or considering pregnancy should be referred to the nearest such centre.

▪ Outcome

The experience of specialist centres is that all women who plan their pregnancies and start diet preconception can maintain excellent metabolic control throughout pregnancy irrespective of their baseline PHE levels [52]. Levels may rise transiently if there is morning sickness or intercurrent illness, but with frequent monitoring, these episodes can be rapidly controlled by a combination of reducing natural protein intake and increasing the dose of amino acid supplements. With morning sickness it is particularly important for women to maintain their calorie intake, in order to prevent catabolism, and to keep taking their supplements. A recent audit showed that if failure of metabolic control was defined as a PHE level above 450 μmol/l for a week or more, over a 2-year period none of 13 women who started diet preconception met the criteria for failure at any time during pregnancy (R. Lachmann, unpublished data 2009).

In practice, after the first trimester it becomes progressively easier to maintain PHE levels within the target range, as protein tolerance increases markedly as the baby grows. For many women who choose to remain on a low-protein diet after delivery, greater protein restriction is required post partum, despite the fact that the target PHE levels are significantly higher than during pregnancy.

Women who only start the diet when they are already pregnant have significantly higher PHE levels at all stages of pregnancy [52]. For the most part, PHE levels can be quickly brought under control. Even when compliance with a strict low-protein diet is poor, PHE levels tend to fall as pregnancy progresses owing to the increased protein tolerance discussed above. There is, nonetheless, a small subgroup of women who are unable to fully comply with the requirements of a low-protein diet and who never obtain satisfactory metabolic control. If they can be admitted for full supervision of their diet, PHE levels invariably come down, but prolonged in-patient stays are neither practicable nor acceptable to the patients. Outcomes of such pregnancies remain poor, particularly in terms of head circumference, birth weight and development. Often successive pregnancies are affected in the same way. It is in the nature of these pregnancies that monitoring of PHE levels is infrequent, but often the absolute levels are not particularly high (<1000 μmol/l for the majority of pregnancies), and there is some evidence that variation in PHE levels is also associated with developmental outcome [52]. For these women, new interventions are desperately required. BH_4, which is licensed for use in pregnancy, may have a valuable role to play here; the high cost of treatment during pregnancy could easily be justified if it led to significant improvements in IQ for the offspring.

Like the neurological damage seen in children with classic PKU, the maternal PKU syndrome is preventable. The key to ensuring optimal, normal pregnancy outcome is for pregnancy to be planned and for dietary treatment to be established prior to conception. This requires all women with HPA to be educated about pregnancy and its management from an early age, with information regularly reinforced; it is critical that all women with PKU are transferred to adult metabolic units so that they can be kept under specialist review throughout their childbearing years.

17.4 HPA and Disorders of Biopterin Metabolism

Disorders of tetrahydrobiopterin (BH_4) associated with HPA and biogenic amine deficiency include GTP cyclohydrolase I (GTPCH) deficiency, 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency, dihydropteridine reductase (DHPR) deficiency and pterin-4a-carbinolamine dehydratase (PCD) deficiency (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).

17.4.1 Clinical Presentation

The condition can present in any of three ways:

1. Asymptomatic, but with raised PHE found following newborn screening; 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 newborn screening 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 newborn screening for HPA or if the PHE level is sufficiently low not to have resulted in a positive screen or required 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. Cerebral atrophy and cerebral calcification can occur in DHPR deficiency. There is a mild (peripheral) form of PTPS associated with HPA but without neurotransmitter deficiency, where there are no neurological symptoms. In PCD deficiency symptoms are mild and transient.

17.4.2 Metabolic Derangement

Disorders of pterin synthesis or recycling are associated with decreased activity of PAH, tyrosine hydroxylase, tryptophan hydroxylase and nitric oxide synthase (◘ Fig. 17.1). The degree of HPA, due to the PAH deficiency, 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 concentrations 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.

17.4.3 Genetics

All disorders are autosomal recessive. Descriptions of the relevant genes and a database of mutations are available on www.BH4.org. 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 [54].

17.4.4 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 newborn screening should have blood DHPR and urine or blood pterin analysis. The interpretation of results is shown in ◘ Table 17.3.

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, 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 [54, 55].

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 [56].

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 (CVB). Analysis of amniotic fluid neopterin and biopterin in the second trimester is available for all conditions. Enzyme analysis can be under-

◘ **Table 17.3.** 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	90-1200	↓↓	↓↓	-	↓	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

taken in fetal erythrocytes or in amniocytes in both DHPR deficiency and PTPS deficiency. GTPCH is only expressed in fetal liver tissue.

17.4.5 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 [58]. It is therefore usually recommended that in DHPR deficiency HPA should be corrected by dietary means and BH_4 should not be given.

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 17.4), which is increased gradually to the recommended maintenance dose. Further dose adjustment depends on the results of CSF HVA and 5HIAA 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 [59], entacapone, a catechol-*O*-methyltransferase (COMT) inhibitor [60] and pramipexole, a dopamine agonist receptor [61].

▪ 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

◘ **Table 17.4.** 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	±	±	-	+

BH4, 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

dose of medication is given. CSF folate should also be measured.

Hyperprolactinaemia occurs as a consequence of dopamine deficiency; measurement of serum prolactin can be used as a method to monitor treatment, with normal values indicating adequate L-dopa replacement, and it has been suggested that this may be a more sensitive marker than the CSF HVA level in deciding on dose adjustment [62].

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 [54, 63-65]. 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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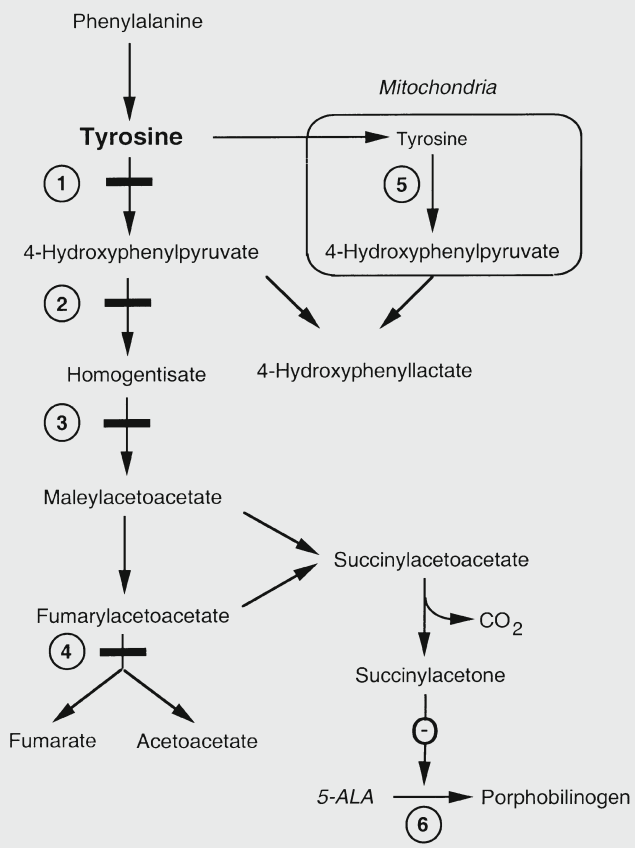
Tyrosine Metabolism

Tyrosine is one of the least soluble amino acids and forms characteristic crystals upon precipitation. It derives from two sources, diet and hydroxylation of phenylalanine (◘ Fig. 18.1). 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, can be 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. 37.1).



◘ **Fig. 18.1.** 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 (5-ALA) dehydratase (porphobilinogen synthase). Enzyme defects are depicted by *solid bars* across the *arrows*

Five inherited disorders of tyrosine metabolism are known, which are depicted in ◘ Fig. 18.1. 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. 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.

18.1 Hereditary Tyrosinaemia Type I (Hepatorenal Tyrosinaemia)

18.1.1 Clinical Presentation

The clinical manifestations of tyrosinaemia type 1 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 1 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 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 1 with NTBC in the last 15 years (► Section 18.1.5) has dramatically altered its natural history.

▪ Hepatic Disease

The liver is the major organ affected in tyrosinaemia 1, 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 1 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 initial 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 1 – 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 micromacronodular type with a variable degree of steatosis. Hepatocyte dysplasia is common, with a high risk of malignant transformation within these nodules [1, 2]. Unfortunately, the differences in size and fat content of the nodules make it difficult to detect malignant changes (► Section 18.1.5).

▪ 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 can become much worse 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 NTBC 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 [5]. Complications include seizures, extreme hyperextension, self-mutilation, respiratory paralysis and death.

▪ Other Manifestations

Cardiomyopathy is a frequent incidental finding, but may be clinically significant [6]. Pancreatic cell hypertrophy may result in clinically significant hyperinsulinism [7].

18.1.2 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, 9]. Disruption of sulfhydryl metabolism is also believed to cause secondary deficiency of two other hepatic enzymes, 4-hydroxyphenylpyruvate dioxygenase and methionine adenosyltransferase, resulting in hypertyrosinaemia and hypermethioninaemia. Additionally, FAA and MAA are alkylating agents and can disrupt the metabolism of thiols, amines, DNA and other important intracellular molecules. 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 an adaptive alteration of gene expression that may predispose to future malignant transformation [10, 11]. In patients who have developed cirrhosis, self-induced correction of the genetic defect and the enzyme abnormality occurs within some nodules [12]. The clinical expression of hepatic disease may correlate inversely with the extent of mutation reversion in regenerating nodules [13]. The mechanisms that underlie the development of hepatocellular carcinoma within nodules are poorly understood.

SA is a potent inhibitor of the enzyme 5-ALA dehydratase. 5-ALA, a neurotoxic compound, accumulates and is excreted at high levels in patients with tyrosinaemia type 1 and is believed to cause the acute neurological crises seen during decompensation [5]. SA is also known to disrupt renal tubular function, haem synthesis and immune function [14-16].

18.1.3 Genetics

Hereditary tyrosinaemia type I is inherited as an autosomal recessive trait. The FAH gene has been localised to 15q23–q25 and more than 40 mutations have been reported [17]. The most common mutation, IVS12+5(g-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, IVS6-1(g-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 [18]; spontaneous correction of the mutation within regenerative nodules may influence the clinical phenotype [13]. 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 [19]. A pseudodeficiency mutation, R341W, has been reported in healthy individuals who have in vitro FAH activity indistinguishable from that in patients with type 1 tyrosinaemia [20]. The frequency of this mutation in various populations is unknown, but it has been found in many different ethnic groups.

18.1.4 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 1. However, very rarely, urine succinylacetone elevation may be absent in mild cases [19]. Other metabolite abnormalities that are suggestive of the diagnosis include elevated plasma levels of tyrosine, phenylalanine and methionine, reduced erythrocyte 5-aminolevulinate 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 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 patients' clinical and biochemical findings.

▪ Newborn Screening

Screening using tyrosine levels alone has been used in the past and has resulted in very high false-positive and false-negative rates [21]. SA is a highly sensitive and specific marker for tyrosinaemia type 1, and assays based on the inhibitory effects of SA on 5-ALA dehydratase, either alone or in combination with tyrosine levels with tyrosine levels, have greatly improved diagnostic accuracy [22]. More recently, screening methods based on the direct measurement of SA in dried blood spots by tandem mass spectrometry have been developed and validated [23], facilitating the potential inclusion of tyrosinaemia type 1 in current newborn screening programmes. Molecular screening is possible in populations in which one or few mutations account for the majority of cases.

▪ Prenatal Diagnosis

If the causative mutations in a pregnancy at risk are known, 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 [24]. When mutation

analysis is not available for prenatal diagnosis, we currently use 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.

18.1.5 Treatment and Prognosis

Historically, tyrosinaemia type I was treated with a tyrosine- and phenylalanine-restricted diet, with or without liver transplantation. In 1992 a new drug, 2-(2-nitro-4-trifluoro-methylbenzoyl)-1,3-cyclohexanedione (NTBC or nitisinone), a potent inhibitor of 4-hydroxyphenylpyruvate dioxygenase, was introduced (◘ Fig. 18.1, enzyme 2). It has revolutionised the treatment of type 1 tyrosinaemia [25] and is now the mainstay of therapy, with more than 600 patients treated worldwide.

- **NTBC (Nitisinone)**

The rationale for the use of NTBC 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-hydroxyphenyl-pyruvate and -lactate concomitantly increase (◘ Fig. 18.1). The Gothenberg Multicentre Study provides the major experience of NTBC treatment in tyrosinaemia type 1 [26]. Over 300 patients have been treated; of these, over 100 have been treated for over 5 years. NTBC acts within hours of administration and has a long half-life of about 54 hours [27]. 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 may not normalise for up to several months after the start of treatment. NTBC is recommended in an initial dose of 2 mg/kg body weight per day in liver failure or 1 mg/kg/day otherwise [26]. Individual dose adjustment is subsequently based on the biochemical response and the aim is a plasma NTBC concentration of >50 μmol/l. Dietary restriction of phenylalanine and tyrosine is necessary to prevent the known adverse effects of hypertyrosinaemia (see tyrosinaemia type II). We currently aim to maintain tyrosine levels between 200 and 400 μmol/l using a combination of a protein-restricted diet and phenylalanine- and tyrosine-free amino acid mixtures.

A small proportion of acutely presenting patients (<10%) do not respond to NTBC treatment; in these patients, coagulopathy and jaundice progress and mortality is very high without urgent liver transplantation. If encephalopathy develops or if prothrombin time does not improve within 1 week, urgent liver transplantation should be considered.

Adverse events of NTBC 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 [26]. The short- to medium-term prognosis in responders appears to be excellent. Hepatic and neurological decompensations are not known to occur on NTBC 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 NTBC.

The risk of hepatocellular carcinoma appears to be much reduced in patients started early on NTBC treatment. In particular, the risk is very low if treatment is commenced before 6 months of age. In patients started on NTBC after 6 months of age, the risk of developing hepatocellular carcinoma increases with the age at which treatment is introduced; if NTBC is introduced after 2 years of age, the risk may not be much different from that in historical controls (◘ Table 18.1). It remains to be determined whether early NTBC treatment can prevent liver cancer in the long term. Studies on the animal mouse models suggest that late hepatocellular carcinoma may occur even if NTBC treatment is started at birth [28]; careful long-term vigilance is therefore necessary in all patients.

The long-term neuropsychological outcome of NTBC-treated patients with tyrosinaemia type 1 is also unclear. Many patients appear to have significant learning difficulties; cognitive deficits affecting performance abilities more than verbal abilities have been found in many patients on psychological testing. As many as 35% of children may have learning difficulties [29]. The aetiology of these cognitive deficits is uncertain; whether they are related to NTBC treatment, high tyrosine levels, low phenylalanine levels or liver failure, or are an intrinsic feature of tyrosinaemia 1 per se, is unknown.

Monitoring of patients on NTBC treatment should include regular blood tests for liver function, blood counts, clotting, plasma PBG synthase activity, 5-ALA, NTBC 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.

Monitoring for hepatocellular carcinoma consists of α-fetoprotein checked every 3 months, in combination with hepatic imaging by ultrasound every 6 months and

Table 18.1. Risk of hepatocellular carcinoma (HCC) in tyrosinaemia type 1

	Reference	Age at start of treatment with NTBC	Number of patients	Patient age (in years) at assessment	Patients developing HCC (%)
Pre-NTBC	[3]	n/a	43	>2	16 (37%)
	[1]	n/a	55	2-12	10 (18%)
Post-NTBC	[26]	<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 nitrisonone

by CT/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 1 and allows a normal unrestricted diet [30, 31]. However, even in optimal circumstances, it is associated with approximately 5-10% mortality and necessitates lifelong immunosuppressive therapy. Therefore, at present liver transplantation in type 1 tyrosinaemia is restricted to patients with acute liver failure who fail to respond to NTBC therapy, and patients with suspected hepatocellular carcinoma. Liver transplantation may be considered for complications of chronic liver disease, such as severe portal hypertension, growth failure and poor quality of life, but these are unusual in the NTBC era.

The long-term impact of liver transplantation on renal disease in tyrosinaemia type 1 is not fully known and relates to the era they were treated in. Prior to NTBC, 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 [30, 32]. Patients pre-treated with NTBC usually have normal renal function at transplant, and this combined with the development of kidney-sparing immunosuppression regimens ensures they have a much improved renal prognosis.

After transplantation, when NTBC is discontinued, renal production results in significantly elevated plasma and urinary SA levels. The functional significance of these findings is unclear, but they may predispose to future renal dysfunction. If residual SA excretion is shown to be pathogenic post transplant NTBC treatment may need to be considered, which would probably necessitate reintroduction of dietary restriction.

■ Dietary Treatment

Before the advent of NTBC therapy, dietary protein restriction was the only available treatment for tyrosinaemia type 1 apart from liver transplantation. Dietary treatment was helpful in relieving the acute symptoms and perhaps slowing disease progression, but it did not prevent the acute and chronic complications, including hepatocellular carcinoma. Currently, dietary therapy alone is not recommended, but is used in conjunction with NTBC therapy to prevent the complications related to hypertyrosinaemia. Ocular and dermatological complications are not believed to occur below plasma tyrosine levels of 800 μmol/l; however, lower levels (200-500 μmol/l) are usually recommended owing to possible effects of hypertyrosinaemia on cognitive outcome. Natural protein intake is restricted to provide just enough phenylalanine and tyrosine to keep plasma tyrosine levels below 500 μmol/l and phenylalanine levels above 40 μmol/l; the rest of the normal daily protein requirement is given in the form of a phenylalanine- and tyrosine-free amino acid mixture. While tyrosine levels are stable there appears to be considerable diurnal variation in phenylalanine levels. The indications for phenylalanine supplementation are currently unclear [33].

■ 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. Addition of vitamin D, preferably 1,25 hydroxy vitamin D_3 or an ana-

logue, may be required to treat rickets. Infections should be treated aggressively.

- **Pregnancy**

To date, no published data on pregnancies in patients on NTBC treatment is available. The experience of pregnancy outcome in tyrosinaemia type 2 suggests that close dietary control would be crucial [34]. Pregnancy is a realistic expectation for the majority of women who have had liver transplantation. 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 1.

18.2 Hereditary Tyrosinaemia Type II (Oculocutaneous Tyrosinaemia, Richner-Hanhart Syndrome)

18.2.1 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 [35]. 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 [36]. 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 [37]. 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.

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 [38].

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.

18.2.2 Metabolic Derangement

Tyrosinaemia type II is due to a defect of hepatic cytosolic tyrosine aminotransferase (◘ Fig. 18.1, enzyme 1). As a result of the metabolic block, tyrosine concentrations in serum and cerebrospinal fluid are markedly elevated. The accompanying increased production of the phenolic acids 4-hydroxyphenyl-pyruvate, -lactate and -acetate (not shown in ◘ Fig. 18.1) may be a consequence of direct deamination of tyrosine in the kidneys, or of tyrosine catabolism by mitochondrial aminotransferase (◘ Fig. 18.1). 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 [39]. 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.

18.2.3 Genetics

Tyrosinaemia type II is inherited as an autosomal recessive trait. The gene is located at 16q22.1-q22.3. Seventeen different mutations have so far been reported in the tyrosine aminotransferase gene [40]. Prenatal diagnosis using mutation analysis on chorionic villus sampling has been reported [41].

18.2.4 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 18.3). Urinary excretion of the phenolic acids 4-hydroxyphenyl-pyruvate, -lactate, -acetate is highly elevated, and *N*-acetyltyrosine and 4-tyramine are also increased. The diagnosis can be confirmed by enzyme assay on liver biopsy or 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 [42].

18.2.5 Treatment and Prognosis

Treatment consists in a phenylalanine- and tyrosine-restricted diet, and the skin and eye symptoms resolve within weeks of treatment [37]. 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 [43]. 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 [34, 38]; however, other untreated pregnancies have been followed by normal fetal outcome [38, 44], 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 [45] treated with a low-protein diet to maintain plasma tyrosine levels of 100-200μmol/l and phenylalanine levels of 200-400μmol/l, a normal fetal and maternal outcome was reported.

18.3 Hereditary Tyrosinaemia Type III

18.3.1 Clinical Presentation

Only 13 cases of tyrosinaemia type III have been described, and the full clinical spectrum of this disorder is unknown [46]. 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 of the described cases have developed signs of liver disease in the long term. 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.

18.3.2 Metabolic Derangement

Tyrosinaemia type III is due to deficiency of 4-hydroxyphenylpyruvate dioxygenase (HPD) (◘ Fig. 18.1, 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-hydroxyphenyl-pyruvate and its derivatives 4-hydroxyphenyl- lactate and 4-hydroxyphenyl-acetate. The aetiology of the neurological symptoms is not known, but they may be related to hypertyrosinaemia, as in tyrosinaemia types 1 and 2.

18.3.3 Genetics

Tyrosinaemia type III follows an autosomal recessive inheritance. The HPD gene has been localised to 12q24-qter, and five mutations associated with tyrosinaemia III have been described. There is no apparent genotype-phenotype correlation; some patients with enzymatically defined HPD deficiency do not have identifiable mutations in the HPD gene [46, 47].

18.3.4 Diagnostic Tests

Elevated plasma tyrosine levels of 300-1300 μmol/l have been found in the described cases at diagnosis. Elevated urinary excretion of 4-hydroxyphenyl-pyruvate, -lactate and -acetate usually accompanies the increased plasma tyrosine concentration. Diagnosis can be confirmed by enzyme assay in liver or kidney biopsy specimens or by mutation analysis.

18.3.5 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 [46]; 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.

18.4 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. 18.1, 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 [48, 49]. 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 1 may be indicated in this situation.

18.5 Alkaptonuria

18.5.1 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 [50].

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. 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 [50]. A high frequency of renal and prostatic stones has also been reported.

18.5.2 Metabolic Derangement

Alkaptonuria was the first disease to be interpreted as an inborn error of metabolism in 1902 by Garrod [51]. It is caused by a defect of the enzyme homogentisate dioxygenase (◘ Fig. 18.1, 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 may have a role in pathogenesis [52].

18.5.3 Genetics

Alkaptonuria is an autosomal recessive disorder. The gene for homogentisate oxidase has been mapped to chromosome 3q2, and over 90 mutations have been identified [53]. The estimated incidence is between 1:250,000 and 1:1,000,000 live births.

18.5.4 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.

18.5.5 Treatment and Prognosis

A number of different approaches have been used in attempts at 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 [54]. Ascorbic acid prevents the binding of ^{14}C-homogentisic acid to connective tissue in rats [55] and reduces the excretion of benzoquinone acetic acid in urine [56]. Administration of the drug NTBC also reduces urinary homogentisate excretion; the concomitant hypertyrosinaemia requires dietary adjustment to prevent ocular, cutaneous and neurological complications [50]. A recent 3-year clinical trial of NTBC has demonstrated a 95% reduction in urine and plasma homogentisic acid [57]. However, despite such an improvement in the biochemical marker of alkaptonuria and subjective patient reports of improvement in symptom control, objective musculoskeletal parameters did not show statistically significant improvement in the treatment cohort. The patients treated with NTBC had 10-fold increases in serum tyrosine levels, but side effects were limited to one report of subepithelial corneal lesion and one patient with raised liver transaminases. Further studies in larger cohorts were suggested.

To date, no adverse effects on pregnancy have been reported patients with alkaptonuria.

18.6 Hawkinsinuria

18.6.1 Clinical Presentation

This rare condition, which has only been described in a few families [58, 59], 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.

18.6.2 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. 18.1, 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.

18.6.3 Genetics

Hawkinsinuria is thought to be a condition allelic to tyrosinaemia type III, and a heterozygous missense mutation predicting an Ala to Thr change at codon 33 (A33T) was found in the same HPD gene in the two patients with hawkinsinuria [60]. 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 [61]. 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.

18.6.4 Diagnostic Tests

Identification of urinary hawkinsin or 4-hydroxycyclohexylacetate by GC-MS is diagnostic [59]. Hawkinsin is a ninhydrin-positive compound, which appears between urea and threonine in ion-exchange chromatography of urine amino acids. Increased excretion of 4-hydroxycy-

clohexylacetate is detected on urine organic acids analysis. In addition to hawkinsinuria there may be moderate tyrosinaemia, increased urinary 4-hydroxyphenylpyruvate and 4-hydroxyphenyllactate, 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 [60, 61] makes molecular diagnosis possible in some cases.

18.6.5 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.

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Branched-chain Organic Acidurias/Acidaemias

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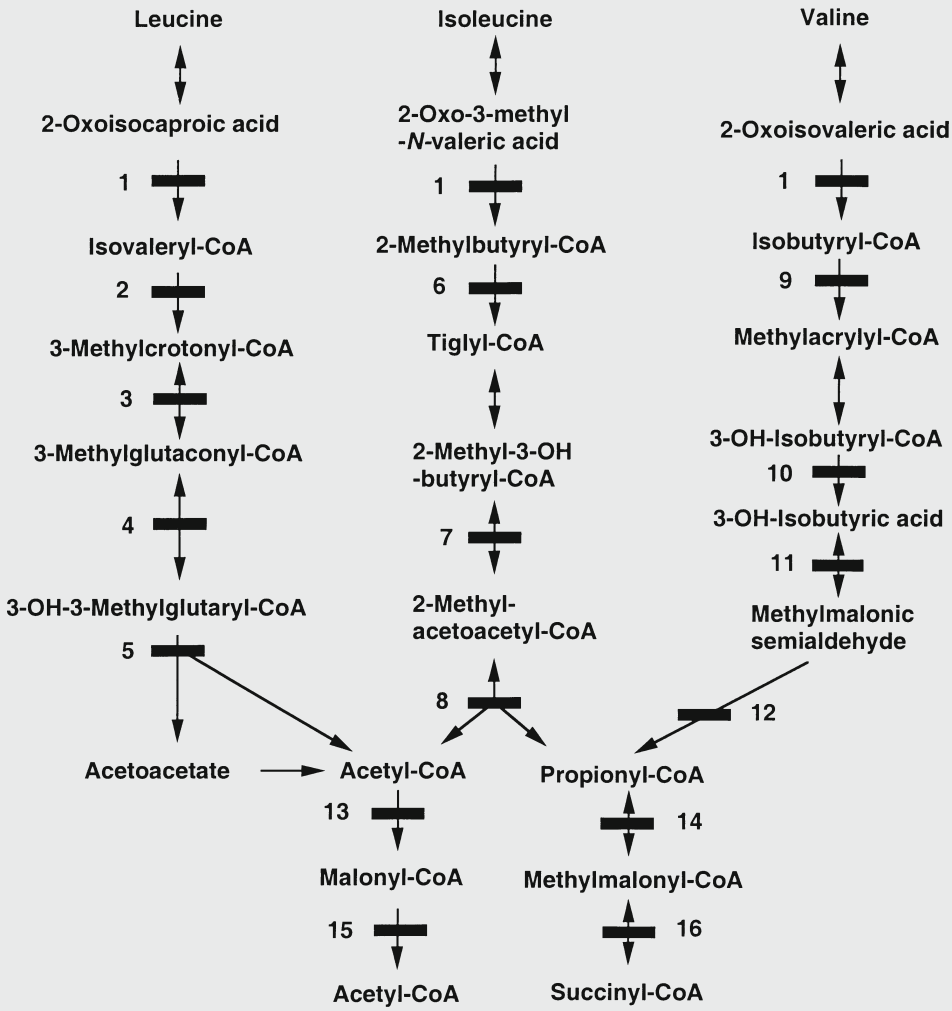
Catabolism of Branched-chain Amino Acids

The three essential branched-chain amino acids (BCAAs), leucine, isoleucine and valine, are initially catabolised by a common pathway (◘ Fig. 19.1). 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 (◘ Fig. 12.2). Subsequently, the degradative pathways of BCAA 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. 19.1.** 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; **7**, 2-methyl-3-hydroxybutyryl-CoA dehydrogenase; **8**, 2-methylacetoacetyl-CoA thiolase; **9**, isobutyryl-CoA dehydrogenase; **10**, 3-hydroxyisobutyryl-CoA deacylase; **11**, 3-hydroxyisobutyric acid dehydrogenase; **12**, methylmalonic semialdehyde dehydrogenase; **13**, acetyl-CoA carboxylase (cytosolic); **14**, propionyl-CoA carboxylase; **15**, malonyl-CoA decarboxylase; **16**, 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). Collectively, the most commonly encountered are maple syrup urine disease (MSUD), isovaleric aciduria (IVA), propionic aciduria (PA) and methyl malonic 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-methylcrotonyl glycinuria, 3-methylglutaconic (3-MGC) aciduria, short-/branched-chain acyl-CoA dehydrogenase deficiency, 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency, isobutyryl-CoA dehydrogenase deficiency, 3-hydroxyisobutyric aciduria, and malonic aciduria. All these disorders can be diagnosed by identifying acylcarnitines and other organic acid compounds in plasma and urine by gas chromatography-mass spectrometry (GC-MS) or tandem MS and all can be detected by newborn screening using tandem MS.

19.1 Maple Syrup Urine Disease, Isovaleric Aciduria, Propionic Aciduria, Methylmalonic Aciduria

19.1.1 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 subjects with an abnormal newborn screening result, have demonstrated the relative frequency of asymptomatic forms.

■ Severe Neonatal-onset Form

■■ General Presentation

The general presentation of this form 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, 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. In contrast, true convulsions 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), and 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 or with the 2,4-dinitrophenylhydrazine (DNPH) test.

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. 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 a urea-

cycle disorder. Moderate hypocalcaemia (<1.7 mmol/l) and hyperlactataemia (3-6 mmol/l) are frequent findings. The physician should be wary of attributing marked neurological dysfunction merely to these. Blood glucose can be normal, reduced, or elevated. When blood glucose level is very high (20 mmol/l) and is associated with glucosuria, ketoacidosis, and dehydration it may mimic neonatal diabetes. Neutropenia, thrombocytopenia, nonregenerative anaemia, and pancytopenia can occur and are frequently confused with sepsis. Among these disorders, IVA is easily recognized by its 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 neurological outcomes.

■ Acute Intermittent Late-onset Form

In approximately one fourth of the patients the disease presents after a symptom-free period, which is commonly longer than 1 year and sometimes even 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 can occur with 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 is that presenting with ketoacidosis, but in exceptional cases this may be absent.

Hypoglycaemia may occur in patients with MSUD, while in the other disorders blood glucose levels are low, normal, or high. Mild hyperammonaemia can be present in IVA, PA, and MMA patients. 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

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. These observations emphasise the importance of complete metabolic investigations in such situations.

■■ 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 humoural 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 leads 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 only presenting symptoms [1].

■ Complications

■■ Neurological Complications

Maple Syrup Urine Disease. Acute cerebral oedema is a well-recognised complication in newborn infants with MSUD and encephalopathy. Brain ultrasonography displays a characteristic pattern that may be of help in the diagnosis [2]. In older patients with metabolic decompensation it may cause brain stem compression and unexpected death, particularly following intensive rehydration [3]; 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 [4, 5]. Acute axonal neuropathy may complicate late-onset decompensation [6].

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, magnetic resonance imaging (MRI) studies indicate cerebral atrophy and delayed myelination [7, 8]. 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 might indicate that aerobic oxidation is persistently impaired from elevated intracellular propionic metabolites [8]. Late-onset optic neuropathy with visual dysfunction is another insidious complication [9].

▪▪ 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 patients older than 10 years [10]. The renal lesion is a tubulo-interstitial nephritis with type-4 tubular acidosis and adaptative changes secondary to the reduced glomerular filtration rate [11]. The course of the disease is usually indolent, but end-stage renal failure may develop, and dialysis and renal transplantation are likely to be necessary by the end of the 2nd decade of life in many patients [12]. If the nephropathy is the complication of a chronic glomerular hyperfiltration secondary to excessive MMA excretion, minimising and deceleration of renal injury may require strict metabolic control.

▪▪ 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 this group of organic acidaemias. It has been the presenting illness in patients with late-onset forms of IVA. The pathophysiological mechanism is 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. Pancreatitis, being defined by inflammation on pancreas imaging, implies specific dietary therapy with a low-fat diet. In contrast, isolated hyperamylasaemia and hyperlipasaemia would normalise with the correction of the metabolic status [14].

▪▪ Cardiomyopathy

Cardiomyopathy is one of the major complications in PA and may be responsible for rapid deterioration or death. 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 cohort, 70% of patients beyond infancy were found to have developed disturbance in cardiac electrophysiology that could contribute to cardiac complications [16]. The mechanism is uncertain but may result from energy deprivation or toxic accumulation. Investigation and follow-up may be useful to prevent irreversible damage and to help in decisions on therapeutic measures, as recovery with renal replacement therapies or with orthotopic liver transplantation has been described in rare cases [15, 17].

19.1.2 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. 19.1, enzyme 1). Like the other 2-ketoacid dehydrogenases, BCKD is composed of three catalytic components (◘ Fig. 12.2): a decarboxylase (E1), composed of E1α- and E1β-subunits and requiring thiamine pyrophos-

phate 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 12). 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.

The enzyme defect 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 is invariably found in the blood of all classic MSUD patients and in those with variant forms, at least in those still without dietary treatment. This compound is endogenously formed and is a diastereomer of isoleucine.

Among the BCAA metabolites, leucine and 2-ketoisocaproic acid appear to be the most neurotoxic. In MSUD, they are always present in approximately equimolar concentrations in plasma, and may cause acute brain dysfunction 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. 19.1, enzyme 2), an intramitochondrial flavoenzyme which, in a similar way to the acyl-CoA dehydrogenases (◘ Fig. 13.1), 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 13).

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. 19.1, enzyme 14), one of the four biotin-dependent enzymes. PCC is a multimeric protein composed of two different sorts of PCC subunits, α- (which bind 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. Low 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 27).

▪ Methylmalonic Aciduria

MMA is caused by a deficiency of methylmalonyl-CoA mutase (MCM; ◘ Fig. 19.1, enzyme 16), a vitamin B_{12}-dependent enzyme. Deficient activity of the MCM-apoenzyme leads to MMA: Because the apomutase requires adenosylcobalamin (AdoCbl), disorders that affect AdoCbl formation cause variant forms of MMA (► Chapter 28).

The deficiency of MCM leads to the 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 vegetarian or suffers from subclinical pernicious anaemia.

- **Secondary Metabolic Disturbances Common to PA and MMA**

The accumulation of propionyl-CoA results in inhibitory effects on various pathways of intermediary metabolism, in increased levels of acylcarnitines (particularly propionyl carnitine) 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 abnormal 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 evoked to explain acute and long-term organ damage [18].

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) mobilisation and oxidation of odd-chain fatty acids during prolonged fasting states. It has been estimated that catabolism of amino acids contributes approximately 50% to the total propionate production, anaerobic gut bacteria, 20% and odd-chain fatty acids, 30% [19]. 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 [12, 20]).

19.1.3 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. 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 suffer from the milder intermediate or intermittent variants. Over 150 different causal mutations scattered among the three *E1α, E1β* and *E2* genes give rise to either classic or intermediate clinical phenotypes [21].

- **Isovaleric Aciduria**

IVA is an autosomal recessive disorder, with extreme clinical variability for reasons that are unknown. Reported mutations in the *IVD* gene are highly heterogeneous, and generally no phenotype/genotype correlation has been established. However, children with IVA diagnosed by newborn screening and carrying a 932C>T mutant allele can exhibit a milder, potentially asymptomatic phenotype [22].

- **Propionic Aciduria**

PA is an autosomal recessive disorder with an incidence of less than 1 in 100,000. PA can result from mutations in the *PCCA* or *PCCB* genes encoding the α- and β-subunits, respectively, of propionyl-CoA carboxylase.

To date, more than 50 different allelic variations in the *PCCB* gene and more than 30 in the *PCCA* gene have been identified in different populations [23, 24]. Following the introduction of the newborn screening programme in Japan a number of infants with an apparently mild phenotype and the Y435C mutation in the *PCCB* gene have been reported. The natural history of this phenotype is not yet clarified [25]. Particularly in PA, knowledge of the phenotype-genotype correlations may provide important information for the prediction of the metabolic outcome and for the implementation of treatments tailored to individual patients.

- **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, respectively, or cobalamin A or B (Cbl A/B) for cofactor defects. To date more than 50 disease-causing mutations in patients with $mut^{0/-}$ MMA have been identified at the *MUT* locus [26]. MMA is an autosomal recessive disorder. The incidence of both benign and severe forms is about 1 in 50,000. Approximately one half to two thirds of patients have a mutase apoenzyme defect; the remaining patients have cobalamin variants. Genes *MMAA* and *MMAB* for the Cbl A and Cbl B complementation groups have been cloned and deleterious mutations in CblA and CblB patient cell lines, identified [27-29]. It is speculated that the *MMAA* gene product is a component of a transporter or an accessory protein that is involved in the translocation of vitamin B_{12} into mitochondria. The gene product of the *MMAB* gene is a cob(I)alamin adenosyltransferase (see ► Chapter 28 for further details).

Newly described MMA variants include defects in succinyl-CoA synthase and methylmalonyl-CoA epim-

erase. Succinyl-CoA synthase catalyses the conversion of succinyl-CoA to succinate in the Krebs cycle. Its deficiency causes mild MMA, variable lactic acidosis, accumulation of succinyl-carnitine and mitochondrial DNA depletion (► Chapter 15). Succinyl-CoA synthase is composed of an α-subunit (encoded by *SUCLG1*) and two β-subunits (encoded by *SUCLA2* and *SUCLG2*). Several patients with different genetic backgrounds have been found to have mutations in *SUCLA2*, but to date only two families have been reported with mutations in *SUCLG1* [30-34]. 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 that of *SUCLA2* mutation.

A deficiency in methylmalonyl-CoA epimerase has been reported in a subject with mild MMA. This defect has a questionable clinical impact [35, 36].

19.1.4 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 [37, 38]. 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 [39-41]. An increased leucine/isoleucine peak in blood spots taken at 24 or 36 h of age requires immediate notification to the sender. The abnormal acylcarnitine found in PA and MMA is propionylcarnitine (C3-carnitine) and that in IVA is isovalerylcarnitine (C5-carnitine) [40].

Enzymatic studies 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 assays of the DNA 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 on mutational analysis.

19.1.5 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.

▪ 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 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 [42].

▪ 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 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 been proved 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 [43].

The prescribed amounts of leucine or natural protein are provided by natural foods. Breast milk or standard infant formula is used in young infants. 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 requirements vary widely and may be greater than normal to ensure that essential amino acids are not degraded to provide energy or nitrogen for the biosynthesis of nitrogenous metabolites. Reduction of energy intake below the individual's requirements results in a decreased growth rate and a metabolic imbalance. The energy requirement is met through natural foods, special amino acid formulas and additional fat and carbohydrates from other sources, including protein-free modular feeds. Distribution of energy intake from protein, carbohydrates and lipids should approach the recommended percentages. The diet must be assessed for minerals, vitamins and trace elements and, if incomplete, supplemented with an appropriate commercial preparation. Hyperosmolarity of formulas should be avoided by offering sufficient fluids.

■ Evaluation of Clinical and Nutritional Status

This comprises regular evaluation of weight, length and head circumference, which should all follow growth percentiles appropriate for the patient's age. Nutritional status is also judged by blood cell count, haemoglobin and haematocrit, plasma protein and albumin, iron and ferritin, evaluation of calcium/phosphate metabolism and plasma amino acid profile. 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. In patients treated with a low-protein diet without an added amino acid mixture, measurement of urea excretion is an easy means to evaluate anabolism [43]. Regular assessment of developmental progress provides the opportunity for psychological support, as social and emotional needs are major elements 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 advised for the reversal of acute metabolic decompensation in symptomatic newborns with the classic form of MSUD [44]. 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 (tube feeding) should be started early and BCAA intake adjusted according to the plasma levels, which are monitored daily until the optimal equilibrium is attained. 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 [3, 45].

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 phenylketonuria. 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 and 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 approximately 50-60% of the leucine intake in healthy newborns. Minimum valine and isoleucine requirements are 200-250 mg/day. Apart from considerable interindividual 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. Occasionally, small amounts of free valine and isoleucine must be added 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 (0.15-0.20 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. Such therapy may prevent metabolic decompensation following major surgery and trauma and can obviate the necessity for extracorporeal toxin removal in critically ill children.

Maternal MSUD. In a woman 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 a healthy infant. 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 [46].

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 [47, 48].

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 [45, 49]. The intellectual outcome is inversely related to how long after birth plasma leucine levels remain above 1 mmol/l and is dependent on the quality of long-term metabolic control [50]. This suggests that inclusion of MSUD in neonatal screening programmes by tandem-mass spectrometry may improve the prognosis in this disease. Normal development and normal intellectual outcome and performance can be achieved at least in prospectively treated patients [3] and if average long-term plasma leucine levels are not more than 1.5-2 times normal [50]. 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 [45]. 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 [3]. Assiduous care is also indicated for patients with variant forms, in order to prevent further ketoacidotic crises after they have been diagnosed and to retain the relatively good prognosis.

■■ 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, who 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.

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 [51]. 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 no longer occur. Growth is normal; intellectual prognosis depends on early diagnosis and treatment and, subsequently, on long-term compliance [52]. According to this, inclusion of IVA into neonatal screening programmes by tandem MS should improve the prognosis. So far (only one pregnancy published) there is no evidence that uncomplicated maternal IVA has any adverse effect on the unborn child [53].

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 find out the natural history.

■■ Propionic Aciduria and Methylmalonic Aciduria

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/day. Thus, emergency treatment of the newborn with MMA, if not complicated by very high ammonia levels, mainly comprises rehydration and promotion of anabolism [54].

When conservative measures with high energy supply are sufficient, hyperammonaemia (especially in PA) may be controlled by use of sodium benzoate and/or carbamoylglutamate [55]. Metabolic decompensation in PA may be complicated by severe lactic acidosis due to thiamine deficiency, requiring vitamin supplementation [56].

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 [42], 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 [12, 42, 43]. 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 [43].

The diet must be nutritionally complete, with adequate energy intake and sufficient vitamins and minerals, in order to save the patient from serious complications associated with poor nutrition. Long fasts should be avoided. In order to prevent fasting at night nocturnal tube feeding may be used 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 state 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 is difficult to feed [12, 42, 43].

Most patients with a late-onset form are easier to manage. Individual protein tolerance can be quite high. Even though their individual tolerance 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 B_{12}. Some late-onset forms (and, more rarely, neonatal-onset forms) of MMA are responsive to vitamin B_{12}; thus, parenteral vitamin therapy, starting with hydroxycobalamin 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-B_{12} responsiveness leads to a prompt and sustained decrease of propionyl-CoA byproducts. 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 B_{12} is either given orally once per day or administered once a week (1000-2000 μg i.m.). In some cases, i.m. hydroxycobalamin therapy can be kept in reserve 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 in this way reducing propionate toxicity [12].

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 [12]. This 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 [12]. 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 [57].

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 in urine [43]. Regular amino acid analysis (all essential amino acids, and in particular isoleucine) is important. Furthermore, methylmalonic acid 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 B_{12} responsive and have mild disease and a good long-term outcome [10, 58, 59]. Conversely, both vitamin B_{12}-unresponsive patients with MMA and those with PA have severe disease and many encephalopathic episodes, mainly due to intercurrent infections [60]. Among all patients with all forms of MMA, mut^0 patients have the poorest prognosis, and vitamin B_{12}-responsive CblA and mut^- patients, the best [10, 58]. Owing to earlier diagnosis and better treatment, outcomes for PA and MMA patients have improved in the last decade [43, 59, 60]. Survival rates into early and mid-childhood can now exceed 70%. However, morbidity, in terms of cognitive development, remains high, with a majority of pa-

tients having DQ/IQ in the mildly to moderately retarded range. With the improved management the frequency of growth retardation has decreased, and now most patients with PA and MMA have growth curves within the normal range [43]. Abnormal neurological signs (mainly movement disorders, chorea, dystonia) continue to increase with age [10, 58-60]. Chronic progressive impairment of renal function is a frequent and serious complication that manifests in older patients with high methylmalonic acid excretion [10, 58]. Renal transplantation is likely to be necessary for many patients with MMA who survive into adolescence [61]. 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 outcome 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 us to draw final conclusions about the effects of newborn screening on long-term outcome [60].

There are only a few reports of female patients with MMA who have carried a pregnancy to term. The outcome was favourable despite high levels of methylmalonic acid in blood and urine [62, 63].

Liver/KidneyTransplantation. In view of the poor long-term prognosis associated with a high risk of complications, liver or combined liver-kidney transplantations have been performed in a growing group of patients of different ages (from early infancy to adulthood) [64, 65]. After successful transplantation most patients have returned to a liberalised diet. A few patients with PA had remission of cardiomyopathy following liver transplantation [15, 17]. Despite sometimes only slight improvement in the levels of circulating propionyl-CoA metabolites, life-threatening episodes of ketoacidosis disappeared or were reduced to some degree. 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 is ample experience that progressive renal failure and neurological dysfunction, including metabolic stroke, are not always prevented. Successful isolated kidney transplantation has been performed in some MMA patients in end-stage renal failure, with a very significant improvement in their metabolic control [66].

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, immunisation, 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, including information on a semi-emergency diet, in which natural protein intakes are 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 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 [42]. 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, applying the same rules as for the treatment of late-onset forms. During this stage of management close metabolic evaluation is advised, as the condition is labile and may deteriorate, requiring adjustment of the therapy. Conversely, if the patient's condition improves quickly normal feeding should be restored 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 means of improving metabolic control and preventing further deterioration.

19.2 3-Methylcrotonyl Glycinuria

19.2.1 Clinical Presentation

The clinical phenotype ranges from neonatal onset with severe neurological involvement and even death to complete lack of any symptoms in adults. On the whole, symp-

tomatic patients present either in the neonatal period or later in childhood. Some infants present with intractable seizures from the 1st days of life, others with feeding difficulties, failure to thrive, and hypotonia within the 1st weeks after weaning, and some have recurrent seizures resulting in microcephaly and developmental delay. Most patients, however, present with a Reye-like syndrome following intercurrent illness or a protein-enriched diet within the first 2 years of life, developing neurological manifestations along with hypoglycaemia, ketoacidosis, hyperammonaemia and very low plasma carnitine. Additional manifestations in late-onset patients include muscular hypotonia, seizures, psychomotor retardation, hemiparesis ('metabolic stroke'), signs of 'metabolic leukodystrophy' and dilated cardiomyopathy. A few adult women diagnosed following newborn screening of their infants have complained of muscle weakness.

19.2.2 Metabolic Derangement

In 3-methylcrotonyl glycinuria, leucine catabolism is blocked by deficiency of 3-methyl crotonyl CoA carboxylase (3-MCC) (◘ Fig. 19.1, enzyme 3). This enzyme is one of the four biotin-containing carboxylases known in humans. Accumulation of 3-methylcrotonylglycine also occurs in multiple carboxylase deficiency, but in contrast to 3-MCC is found together with lactic acid and derivatives of propionyl-CoA (► Chapter 27).

Owing to the enzyme block, 3-methylcrotonyl-CoA and 3-methylcrotonic acid accumulate. Most of the accumulated acyl-CoA is conjugated with glycine to form 3-methylcrotonylglycine (MCG). In contrast, acylation of 3-methylcrotonyl-CoA with 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. 3-Hydroxyisovalerylglycine has not been found in this condition. However, acylation with carnitine leads to the formation 3-hydroxyisovaleryl carnitine, which is the major abnormal acylcarnitine found in plasma and dried blood by tandem MS techniques.

19.2.3 Genetics

3-MCC is a heteromeric enzyme consisting of α- (biotin-containing) and β-subunits. 3-MCC deficiency results from loss of function mutations in the *MCCA* and *MCCB* genes encoding these subunits. The mutations can be classified into two groups, denoted CGA and CGB. More than 50 mutations have been identified in both genes [67]. 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 the clinical phenotypes, suggesting that there are other unknown cellular and metabolic factors that affect the resulting phenotypes. Although most patients are compound heterozygotes or homozygotes, some are heterozygotes with a dominant negative allele [68]. The introduction of tandem MS into newborn screening has revealed an unexpectedly high incidence 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 [39, 40].

19.2.4 Diagnostic Tests

The diagnosis relies on a characteristic urinary profile of organic acids, with huge excretion of 3-HIVA and 3-methycrotonylglycine and without the lactate, methylcitrate, and tiglylglycine found in multiple carboxlase deficiency (MCD). 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 diagnostic for 3-MCC deficiency, since it is not found in IVA. In other disorders, such as MCD, propionylcarnitine (3C) is also seen, and in 3-hydroxy-3-methylglutaryl CoA lyase deficiency glutarylcarnitine is the major finding (► Chapter 3).

Since family studies and newborn screening have identified a number of totally asymptomatic siblings and mothers with MCC deficiency, it is advisable to search in any affected family for other MCC-deficient subjects by analysis of the acylcarnitine profile in blood and organic acids in urine.

19.2.5 Treatment and Prognosis

Long-term treatment of symptomatic infants based on a mildly protein-restricted diet (meeting the recommended requirements) results in a general improvement and a reduction in the number of exacerbations. It is effective in lowering the abnormal excretion of organic acids which, however, never disappears.

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. Family studies and newborn screening have identified a number of totally asymptomatic siblings and mothers with 3-MCC deficiency who have very low carnitine concentrations in blood and have never had any treatment, so that the need for treatment must be doubted. The poor prognosis described in early-onset forms presenting as neonatal seizures could be due to late diagnosis and treatment. In acute late-onset forms presenting as Reye-like syndrome, all but one patient fully recovered. A consensus protocol to assist clinicians in the diagnosis and management of screen-positive newborns for 3-MCC deficiency has been proposed [69](► Chapter 3).

19.3 3-Methylglutaconic Aciduria

3-Methylglutaconic aciduria is found in a number of inborn errors of metabolism, only one of which is associated with a defect in leucine metabolism (for review see [70]). However, for completeness the other disorders are also considered here.

3-Methylglutaconic Aciduria Type I. 3-Methylglutaconic aciduria type I (3-MGA type I) 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. 19.1, enzyme 4). Defective activity leads to 3-MGC aciduria type I, which 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 type I from the other secondary types. 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 the *AUH* gene have been identified [71, 72]. No clear therapeutic regimen has been described. Carnitine supplementation may have beneficial effects.

3-Methylglutaconic aciduria type I must be distinguished from many other conditions associated with 3-MGC aciduria, which include Barth syndrome (3-MGA type II), Costeff optic atrophy syndrome (3-MGA type III) and disorders of unknown origin, summarised as 3-MGA type IV.

3-Methylglutaconic Aciduria Type II (Barth Syndrome). This X-linked disorder, characterised by dilated cardiomyopathy, skeletal myopathy, neutropenia and mitochondrial respiratory chain dysfunction, is considered in ► Chapter 35.

3-Methylglutaconic Aciduria Type III. 3-MGA type III (Costeff optic atrophy syndrome) has mostly, but not exclusively, been reported in Iraqi Jewish individuals. It is a neuro-ophthalmological syndrome that consists of early-onset bilateral optic atrophy and later-onset spasticity, extrapyramidal dysfunction and cognitive deficits. 3-Methylglutaconic and 3-methylglutaric acid excretion are increased. The disease is caused by mutations in the *OPA3* gene. The high prevalence of this allele in the Iraqi Jewish population (allele frequency of 1 in 10) suggests a founder effect. [73]. Although the majority of *OPA3* mutations are associated with recessive disease, autosomal dominant inheritance has also been reported.

3-Methylglutaconic Aciduria Type IV. There is a relatively large and heterogeneous group of patients with 3-MGC aciduria, who suffer from variable, multisystem diseases and cannot be classified as having type MCG aciduria types I, II, III or V (3-MGC aciduria type IV, unspecified diseases). Some have been described with respiratory chain disorders [74]. A group of these patients may have quite a well-defined and similar clinical presentation without any clear enzymatic defect [75]. Conversely, mutations in the *TMEM70* gene have been observed in patients with 3-MDC aciduria, hypertrophic cardiomyopathy and mitochondrial ATP synthase deficiency [76].

3-Methylglutaconic Aciduria Type V. 3-MGA aciduria type V, or dilated cardiomyopathy with ataxia (DCMA) syndrome, presents with an early-onset dilated cardiomyopathy with conduction defects and nonprogressive cerebellar ataxia. The disorder, which has been described in 18 patients belonging to the Canadian Dariusleut Hutterite population, is also associated with testicular dysgenesis and growth failure [77]. There are 5- to 10-fold increases in both plasma and urine 3-MGC and 3-MG acid. A mutation in the *DNAJC19* gene, which codes for a protein believed to act as molecular chaperone in the inner mitochondrial membrane, was identified by homozygosity mapping.

19.4 Short-/Branched-chain Acyl-CoA Dehydrogenase Deficiency

Isolated 2-methylbutyrylglycinuria, caused by 2-methylbutyryl-CoA dehydrogenase deficiency (MBD) and encoded by the *ACDSB* gene (◘ Fig. 19.1, enzyme 6), is an autosomal recessive disorder of isoleucine metabolism [78]. 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).

19.5 2-Methyl-3-Hydroxybutyryl-CoA Dehydrogenase Deficiency

Only a few patients with 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD) deficiency 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. All 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.

MHBD deficiency (◘ Fig. 19.1, enzyme 7) is a defect in the degradation of isoleucine and branched-chain fatty acids. 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 the X-chromosomal *HADH2* gene. 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. 19.1, enzyme 8), also known as 3-ketothiolase or T2, is discussed in ► Chapter 14.

19.6 Isobutyryl-CoA Dehydrogenase Deficiency

The mitochondrial enzyme isobutyryl-CoA dehydrogenase (IBD) catalyses the third step in the degradation of valine (◘ Fig. 19.1, enzyme 9). It is encoded by the *ACAD8* gene [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 cardiomyopthy. Other patients have been identified following the expansion of newborn screening [41, 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.

19.7 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, *(MMSDH)* gene) (◘ Fig. 19.1, enzyme 12) [83] and 3-hydroxyisobutyryl-

CoA deacylase deficiency (◘ Fig. 19.1, enzyme 10) have been identified [84].

19.8 Malonic Aciduria

MA is a rare condition, with fewer than 30 cases reported. Deficient malonyl-CoA decarboxylase (MLYCD) is usually expressed in fibroblasts or leukocytes, and various mutations have been reported in the *MLYCD* gene [85].

19.8.1 Clinical Presentation

A neonatal form has been described in some patients, who displayed progressive lethargy, hypotonia, hepatomegaly associated with metabolic acidosis, and mild hyperammonaemia, variously associated with hypoglycaemia and/or hyperlactacidaemia. Cardiac failure due to cardiomyopathy could be present at birth.

In the late-onset forms, most patients present acute metabolic episodes secondary to intercurrent infections. Some of these patients could be 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 has been present in about 40% of identified patients.

19.8.2 Metabolic Derangement

Malonic aciduria is due to deficiency of MLYCD (◘ Fig. 19.1, enzyme 15). The physiological role of this cytosolic enzyme could be in the regulation of cytoplasmic malonyl-CoA abundance and, thus, of mitochondrial fatty acid uptake and oxidation. Patients with MLYCD deficiency display a number of phenotypes that are reminiscent of mitochondrial fatty acid oxidation disorders [85]. 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.

19.8.3 Genetics

MLYCD deficiency is an autosomal recessive disorder. More than 20 mutations in the *MLYCD* gene have been reported. No hotspot mutations have been identified. No phenotype-genotype relationship was detected, and siblings may have different presentation/s [85]. Anaother rare disorder presenting with malonic and methylmalonic aciduria has been recently elucidated as secondary to ACSF 3 deficiency, a member of the acyl-CoA synthetase family (86).

19.8.4 Diagnostic Tests

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.

Total and free carnitine concentrations in plasma are low. Documented accumulation of malonylcarnitine would allow tandem mass spectrometry screening of newborn blood spots. MLYCD has been found to be reduced in cultured fibroblasts and/or leukocytes of most defective cell lines, with residual activity less than 10% of control. Patients with normal enzyme activity in fibroblasts have a similar disorder, with mutations in the *MLYCD* gene [85].

19.8.5 Treatment and Prognosis

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 [87]. 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.

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Disorders of the Urea Cycle and Related Enzymes

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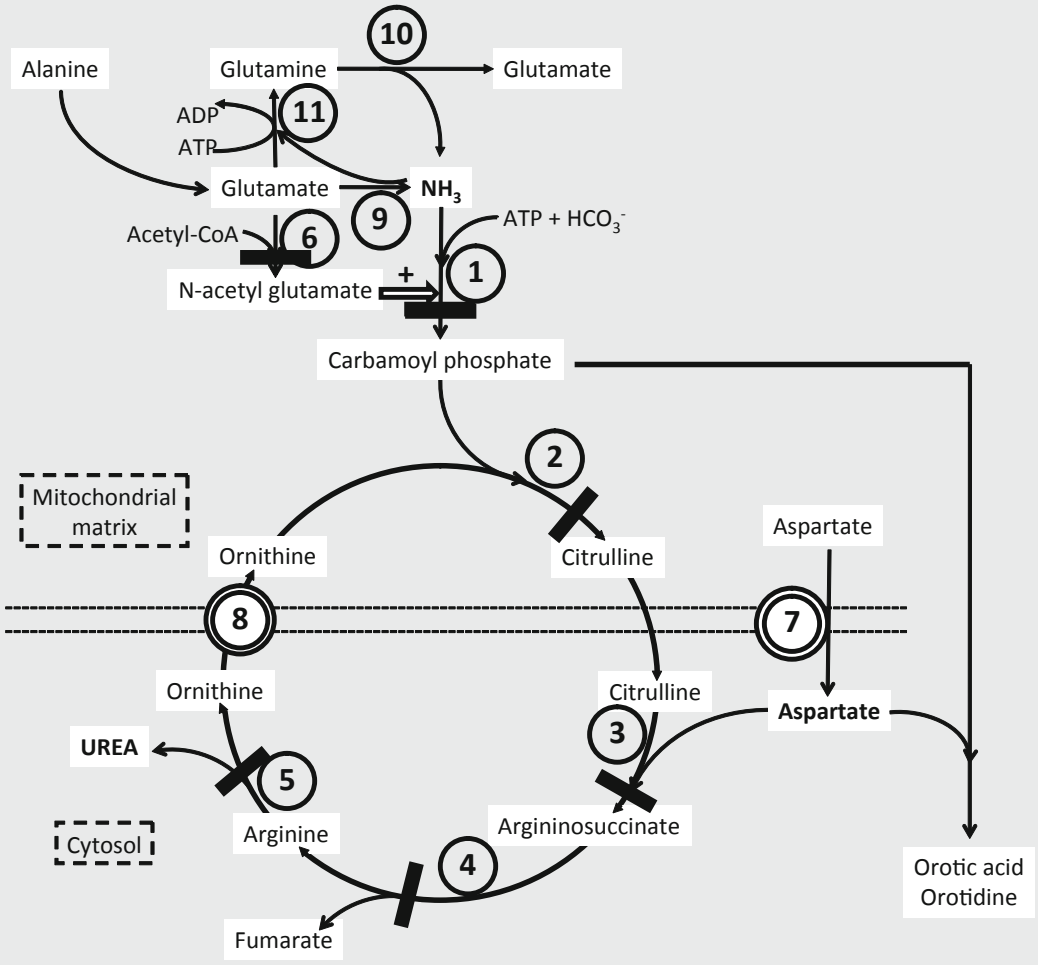
The Urea Cycle

The urea cycle (◘ Fig. 20.1) is composed of six enzymes; three are located in the mitochondrial matrix (carbamoyl phosphate synthetase 1 [CPS1], ornithinine transcarbamoylase [OTC] and *N*-acetylglutamate synthetase [NAGS]), and three are located in the cytosol (argininosuccinate synthetase [ASS], argininosuccinate lyase [ASL] and arginase). Transfer of metabolites across the mitochondrial membrane is accomplished by two carriers: the mitochondrial aspartate-glutamate carrier (citrin) and the mitochondrial ornithine transporter, whereas citrulline diffuses freely. The urea cycle constitutes the main pathway for the disposal of potentially highly toxic ammonia (NH_3), derived from the nitrogen from amino acids, in the form of urea. One turn of the cycle results in the disposal of two nitrogen atoms: one from NH_3 and a second from aspartate. Urea diffuses from the cells into the blood, from which it is cleared into the urine. Although most urea synthesis occurs in the liver, several of its enzymes are expressed in other tissues. Formation and utilisation of urea cycle intermediates therefore also proceed in other organs. In particular, circulating citrulline is mostly synthesised in the intestines, and it is utilised by the kidneys to produce arginine. Additional information with respect to the urea cycle is provided in ► Sections 20.2.1 and 20.2.2.



◘ **Fig. 20.1.** The urea cycle, its directly related transporters and key metabolites;**1**, carbamoyl phosphate synthetase 1 (CPS1); **2**, ornithine transcarbamoylase (OTC); **3**, argininosuccinate synthetase (ASS); **4**, argininosuccinate lyase (ASL); **5**, arginase; **6**, *N*-acetyl glutamate synthetase (NAGS); **7**, mitochondrial aspartate-glutamate carrier (citrin); **8**, mitochondrial ornithine transporter; **9**, glutamate dehydrogenase; **10**, glutaminase; **11**, glutamine synthetase. Enzyme defects are indicated by *solid bars* across the *arrows*

Six inherited enzyme defects of the urea cycle are known. These are the deficiencies of *carbamoylphosphate synthetase (CPS), ornithine transcarbamoylase (OTC), argininosuccinate synthetase (ASS), argininosuccinate lyase (ASL), arginase* and *N-acetylglutamate synthetase (NAGS)*. Defects of transport of urea cycle intermediates have also been described: *aspartate-glutamate carrier (citrin) and mitochondrial ornithine transporter in HHH syndrome*. All these defects are characterised by hyperammonaemia and disordered amino acid metabolism. Their presentation is highly variable: in the newborn period there is usually an overwhelming illness that rapidly progresses from poor feeding, vomiting, lethargy or irritability and tachypnoea to convulsions, coma and respiratory failure. In infancy, the symptoms are less severe and more variable. Poor developmental progress, behavioural disturbances, hepatomegaly and gastrointestinal symptoms are common. Children and adults frequently have a chronic neurological illness characterised by variable behavioural problems, confusion, irritability and cyclic vomiting. Still, during any metabolic stress the patients may become acutely unwell. Arginase deficiency has more specific symptoms, such as spastic diplegia, dystonia, ataxia and convulsions. All urea cycle disorders have an autosomal-recessive mode of inheritance except OTC deficiency, which is X-linked.

20.1 Clinical Presentation

The classic presentation of patients with an inherited defect in one of the enzymes of the urea cycle is that of a rapidly progressive intoxication of the central nervous system (CNS) in the newborn period, with poor feeding, lethargy, irritability and tachypnoea. If untreated, patients with this acute presentation will develop seizures, coma, respiratory insufficiency and vasomotor instability. Death may follow within days.

The phenotypic spectrum of urea cycle defects varies considerably, however, and patients may present at different ages and with a remarkable variety of symptoms. In addition, some patients may present with clinically significant signs and symptoms, including developmental delay and severe liver disease, without elevation of the plasma ammonia concentration.

Hepatomegaly and signs of liver insufficiency may occur in patients with urea cycle defects, but these are generally mild and can be completely absent, even in patients with a severe, life-threatening, metabolic derangement.

20.1.1 Neonatal Presentation

A urea cycle defect resulting in CNS intoxication due to hyperammonaemia in the neonatal period constitutes one of the most acute, devastating, and life-threatening metabolic crises in the field of inborn errors of metabolism. Immediate metabolic investigations to elucidate the underlying defect and emergency treatment aimed at a rapid decrease of ammonia levels should both be initiated at the same time.

Patients are generally born after an uneventful pregnancy and delivery, and the first few days of life are often unremarkable. This short symptomless period is due to: (1) the anabolic state of the unborn child owing to an ample supply of energy via the placenta and (2) the removal of any excess ammonia from the unborn child by the mother via the placenta during intrauterine life.

The metabolic deterioration seen during the 1st week of life is most probably triggered by the physiological period of mild starvation during the early days of life after birth or by high protein feedings initiated several days after birth.

The first clinical symptoms are generally poor feeding and lethargy, followed by irritability, vomiting, tachypnoea and hypothermia. Blood gas analysis in patients with hyperammonaemia may reveal respiratory alkalosis, which is caused by a direct stimulation of the respiratory centre by ammonia. However, in severely ill patients seen later, metabolic and respiratory acidosis resulting from cardiovascular failure and respiratory depression may replace the initial alkalosis.

Without appropriate treatment, seizures, deep coma and vasomotor instability rapidly follow. A misdiagnosis of sepsis is often made, despite the absence of risk factors for infection and despite a normal C-reactive protein (CRP) level and a normal white blood cell count. Unfortunately, the plasma ammonia concentration is often not determined until late in the diagnostic work-up. As the prognosis of a patient with neonatal hyperammonaemia due to a urea cycle defect is directly related to both the level of the plasma ammonia concentration and the duration of hyperammonaemia, immediate diagnosis and initiation of treatment is essential. If left untreated, most children affected will die of cardiorespiratory failure and cerebral insufficiency caused by cerebral oedema or haemorrhage. Unfortunately, even patients who have been rapidly diagnosed and received adequate emergency therapy often experience severe brain damage resulting in significant mental and motor deficits.

20.1.2 Presentation in Infants

Inborn errors of the urea cycle can also present with a less acute phenotype, following an uneventful neonatal period. This late presentation can be due to a combina-

tion of factors: (1) the presence of less severe mutations resulting in significant residual enzyme activity and (2) external circumstances, such as absence of prolonged fasting, protein overloading and protein catabolism. As a result, symptoms are less acute, more variable and sometimes intermittent. Symptoms include failure to thrive, anorexia, frequent, cyclic vomiting, developmental delay, behavioural disturbances with listlessness and aggression, headaches and ataxia. However, infants may also suffer from acute episodes with encephalopathic crises, often provoked by an infection or any other state leading to protein catabolism, such as surgical procedures and prolonged fasting although a trigger for the metabolic derangement is not always obvious.

In patients with a less acute presentation there can be a lengthy diagnostic delay, owing to the unspecific nature of the signs and symptoms. Metabolic studies, including plasma ammonia levels and profiling of plasma amino acids and urinary organic acids, are often only done after exclusion of numerous other conditions or after the onset of overt encephalopathy, including progressive developmental delay.

20.1.3 Presentation in Older Children and Adults

Although (sub)acute deterioration of neurological functions, followed by coma, due to a urea cycle defect may occur in patients of all ages, clinical signs and symptoms in older patients are commonly characterised by a more chronic neurological disease with developmental delay, pyramidal tract lesions, psychiatric symptoms or liver disease.

- **Recurrent Episodes with Encephalopathy**

Older children and adults may have recurrent episodes of encephalopathy, characterised by lethargy, anorexia, headaches, vomiting, ataxia, irritability and confusion, often with behavioural disturbances. In addition, focal neurological symptoms with hemiplegia and cortical blindness mimicking strokes have been reported. Between these episodes patients may be completely devoid of signs and symptoms, complicating the diagnostic work-up.

- **Chronic Encephalopathy**

Developmental delay, sometimes with behavioural abnormalities, may be the only symptom in some patients. This attenuated presentation, without episodes of acute metabolic derangement, is observed in patients with argininosuccinate lyase (ASL) deficiency, but has also been reported in attenuated forms of other urea cycle defects.

- **'Cerebral palsy'-like Presentation**

Progressive spastic tetraplegia is often the major symptom in patients with arginase deficiency. Patients with arginase deficiency may also display seizures, developmental delay and failure to thrive, in addition to the more classic CNS intoxication attributable to hyperammonaemia. The high arginine levels have been implicated in the pathogenesis of the pyramidal tract lesions, but this is not substantiated by clear evidence.

- **Liver Disease**

Although moderate elevations of transaminases with decreased liver functions may be observed during metabolic derangement in all urea cycle defects, significant liver disease is generally only seen in patients with ASL deficiency, citrin deficiency and, to a lesser extent, ornithine transcarbamoylase (OTC) deficiency. Some patients with ASL deficiency may develop liver fibrosis progressing to liver insufficiency. The mechanism(s) causing hepatopathy and fibrosis in ASL deficiency are unclear, but toxicity of argininosuccinate and disturbed nitric oxide (NO) synthesis have been suggested [1,2].

The neonatal presentation of a deficiency in the glutamate-aspartate carrier (citrin) is characterised by neonatal intrahepatic cholestasis with increased transaminases, biochemically and clinically not resembling a urea cycle defect, and is referred to as 'neonatal intrahepatic cholestasis associated with citrin deficiency'(NICCD). Patients who recover from this severe neonatal liver disease may at a later age develop symptoms of CNS intoxication related to hyperammonaemia in addition to failure to thrive and gastrointestinal symptoms (citrullinaemia type II, CTLN2). As patients with citrin deficiency may deteriorate rapidly when treated with a high-carbohydrate, low-protein, diet, this condition needs to be considered when one is faced with a patient with liver disease and hyperammonaemia. Although citrin deficiency appears to be more prevalent in Japan and East Asia, it has now been reported as a pan-ethnic disease [3].

- **Trichorrhexis Nodosa**

Abnormally brittle hair due to trichorrhexis nodosa has been regularly reported in patients with ASL deficiency. This is probably caused by chronic arginine deficiency and improves on arginine supplementation.

20.1.4 Newborn Screening

Some of these disorders, especially ASS, ASL, arginase and citrin deficiencies, can be detected by newborn screening. In these situations, patients who are still asymptomatic

but who may be at risk of developing hyperammonaemia later, will be detected and preventive treatment may be started with an improvement of outcome [4].

20.2 Metabolic Derangement

20.2.1 The Urea Cycle, Its Connections and Regulation

The first step of the urea cycle (◘ Fig. 20.1) is catalysed by mitochondrial carbamoyl phosphate synthetase 1 (CPS1). This is a different enzyme from cytosolic CPS2. which catalyses the first step of pyrimidine synthesis (◘ Fig. 36.3). CPS1 requires *N*-acetylglutamate as its obligatory allosteric activator. *N*-acetylglutamate is formed by the condensation of glutamate with acetyl-CoA catalysed by *N*-acetylglutamate synthetase (NAGS). NH_3 is formed from glutamate by glutamate dehydrogenase and from glutamine by glutaminase. Both amino acids are also linked by glutamine synthetase and may function as a temporary buffer for excess nitrogen. The aspartate required for the synthesis of argininosuccinate is provided by transamination. Aspartate is also a precursor of purines and pyrimidines, including orotic acid and orotidine, via CPS2 (◘ Fig. 36.3). The hydrolysis of argininosuccinate produces arginine, a precursor of the synthesis of creatine (◘ Fig. 16.1) and of the vasodilator nitric oxide (NO) and fumarate, an intermediate of the tricarboxylic (TCA) cycle.

The regulation of the urea cycle is still not fully understood, and the complex compartmentalisation of its enzymes still obscures full understanding. Recently, it has been shown that several mitochondrial enzymes are regulated by sirtuins, a highly conserved family of proteins involved in mitochondrial energy production, apoptosis and signalling, and regulated by the nutritional state (protein intake and catabolism, fasting). Among these, sirtuin 3 and 4 can regulate glutamate dehydrogenase and sirtuin 5, CPS1, the latter by a coordinated mechanism of acetylation/deacetylation [5, 6].

20.2.2 Sources of Ammonia and Inter-organ Fluxes

Alanine and glutamine, which are produced by muscle metabolism, are the most important sources of nitrogen for disposal. Alanine is formed by transamination of pyruvate, which is produced by muscle glycolysis. It is transported to the liver, where it serves as substrate for gluconeogenesis. During this process glutamate is formed, presenting NH_3 for disposal via the urea cycle. Ammonia is also directly produced in the gut by urease-positive bacteria. Infection, fever and fasting, all provoking protein catabolism, result in an increased supply of ammonia, which may induce hyperammonaemia in the case of a defect of the urea cycle. Finally, urea synthesis is directly related to nitrogen intake, and amino acids derived from ingested protein not needed for biosynthetic reactions constitute an important source of ammonia and urea.

Plasma citrulline reflects the balance between its synthesis, which is almost exclusively from the activity of intestinal CPS1 and OTC, and its utilisation by renal ASS and ASL to form arginine. This explains why plasma citrulline is decreased in intestinal failure [7, 8] and increased in renal failure [9], and why abnormal citrulline levels often persist after liver transplantation in urea cycle disorders [10].

20.2.3 CNS Toxicity in Urea Cycle Defects

Severe developmental delay with a wide range of other neurological sequelae may result from an inherited disorder of the urea cycle. The pathophysiological substrate of brain disease in hyperammonaemia is complex and includes cortical atrophy, lesions in the basal ganglia, delayed myelination and cystic changes.

The primary cause of NH_3 toxicity to the brain is also complex [11]. High concentrations of ammonia result in astrocyte swelling and loss of neuronal function. A number of secondary reactions contribute to the severe CNS toxicity. These secondary metabolic changes include increased glutamine, which may result in osmotic brain swelling, arginine deficiency and, as a consequence of arginine deficiency, a decrease in NO and creatine synthesis. As a result of these complex secondary neurotoxic mechanisms, fluctuating clinical signs and symptoms in patients with urea cycle defects do not always correlate well with plasma ammonia levels. As the developing CNS is most vulnerable for these toxic effects, urea cycle defects presenting during the neonatal or early infantile period will produce the most devastating irreversible brain damage. The extent of CNS damage correlates with both severity and duration of the hyperammonaemia. In general, plasma ammonia levels in excess of 250-500 µmol/l during the neonatal or infantile period are correlated with irreversible neurological damage.

Cerebral imaging, using MRI and diffusion tensor imaging (DTI), may be helpful to determine the extent of cerebral damage and predict the prognosis [12]. An encephalopathic (slow wave) EEG pattern may be observed during hyperammonaemia.

20.3 Genetics of Urea Cycle Defects

The genes for all the enzymes of the urea cycle, including NAGS, and of the relevant transporters, have now been fully mapped and sequenced (◘ Table 20.1). OTC deficiency, the most common urea cycle disorder, is X-linked; all other disorders are autosomal recessive. As a consequence of modern rapid sequencing techniques, direct mutation analysis has replaced enzymatic testing in liver biopsies in most centres. Many different private mutations have been reported in the genes coding for the urea cycle defects, which may hamper the interpretation of detected sequence variants. Over 400 different mutations have been reported for OTC deficiency. However, mutations are only found in approximately 80% of patients, suggesting frequently occurring mutations in regulatory domains [13].

In some females with suspected OTC carrier status, unstressed metabolic studies, including plasma ammonia, amino acids and urinary orotic acid, may repeatedly give normal results. The oral allopurinol loading test, which aims to inhibit the final step in uridine synthesis, thereby increasing the production of orotic acid and orotidine in patients with a increased production of carbamoyl phosphate, may detect these otherwise biochemically normal patients. However this test lacks sensitivity and

◘ **Table 20.1.** The most important diagnostic findings in defects of the urea cycle or related transporters. Birth prevalences are estimated on the basis of several reports and vary between countries and ethnic groups

Disorder	Alternative name(s)	Plasma amino acid concentrations	Urine orotic acid and orotidine	Tissue for enzyme diagnosis	Gene	Estimated birth prevalence
N-acetyl glutamate dehydrogenase deficiency	NAGS deficiency	↑ Glutamine ↑ Alanine	Normal	Liver*	NAGS	Very rare
Carbamoyl phosphate synthetase deficiency	CPS1 deficiency	↑ Glutamine ↑ Alanine ↓ Citrulline ↓ Arginine	Normal	Liver*	CPS1	1 : 65,000
Ornithine transcarbamoylase deficiency	OTC deficiency	↑ Glutamine ↑ Alanine ↓ Citrulline ↓ Arginine	↑↑	Liver*	OTC	1 : 15,000
Argininosuccinate synthetase deficiency	ASS deficiency Citrullinaemia type 1	↑ Glutamine ↑ Alanine ↑↑Citrulline ↓ Arginine	↑	Liver or fibroblasts	ASS1	1 : 60,000
Argininosuccinate lyase deficiency	ASL deficiency Argininosuccinic aciduria (ASA)	↑ Glutamine ↑ Alanine ↑ Arginino succinate ↓ Arginine	↑	Liver, fibroblasts or RBCs	ASA	1 : 70,000
Arginase deficiency	Hyperargininaemia	↑ Arginine	↑	Liver or RBCs	ARG1	Very rare
Aspartate-glutamate carrier deficiency	Citrullinaemia type 2 (Citrin deficiency)	↑ Glutamine ↑ Alanine ↑Citrulline** ↓ Arginine	↑	- (mutation analysis)	SLC25A13	Very rare
Ornithine transporter deficiency	Hyperammonaemia, hyperornithinaemia, homocitrullinaemia syndrome HHH syndrome	↑ Ornithine ↑ Homocitrulline	↑	- (mutation analysis)	SLC25A15	Very rare

* Liver biopsy for enzyme analysis can now generally be avoided by confirming the diagnosis by mutation analysis. ** Citrulline can be variable, and even in the normal range outside the neonatal period

specificity [14]. Protein and alanine loading should be discouraged as this may result in hyperammonaemia. Mutation analysis is the most appropriate method for the detection of heterozygous OTC females and hemizygotic asymptomatic males responsible for male-to-female transmission.

20.4 Prenatal Diagnosis

As the genes for all enzymes of the urea cycle and its related transporters have now been mapped and sequenced, prenatal diagnosis can best be done by mutation analysis in chorionic villous samples.

Genetic counselling in OTC deficiency is complicated by its inheritance as a partially dominant X-linked disorder, with over 10% of female carriers being symptomatic [15]. Prediction of the clinical phenotype in female OTC carriers is hindered by the process of random X-inactivation. However, the prognosis for females is generally good and will probably be better if detected before the onset of symptoms.Identification of informative polymorphisms may help in prenatal diagnosis if mutation analysis in the proband has failed to demonstrate a disease-causing mutation.

Preimplantation genetic diagnosis has been reported for OTC deficiency [16] and may well also be an alternative approach for other urea cycle defects.

If mutation analysis has not been performed in a proband, or if genetic analysis has proved uninformative, direct enzyme analysis of ASS, ASL and arginase can be performed in chorionic villous biopsies. For those enzymes not expressed in chorionic villi, fetal liver biopsy can be an alternative.

20.5 Diagnostic Tests and Differential Diagnosis

Plasma ammonia is the most important biochemical analyte if a urea cycle defect is suspected, and ammonia should be measured in all newborn and infants with unexplained signs and symptoms such as lethargy, poor feeding, irritability and vomiting. In older children and adults with unexplained, sometimes episodic, encephalopathy, plasma ammonia should preferably be measured during a period of clinical deterioration. In general, there is no need for studies in arterial blood as venous sampling is sufficient to detect the presence of significantly elevated ammonia levels. For reliable determination of ammonia concentration, storing the sample on ice and immediate transfer to the lab are important.

Cut-off levels for significant hyperammonaemia are related to age, analytical methods and sample techniques. Normal plasma ammonia levels are <50 µmol/l in adults and <100 µmol/l in neonates. In general, levels >100 µmol/l in older children and adults and >150 µmol/l in neonates and infants warrant immediate further studies to check for the possibility of a urea cycle defect. However, most patients with acute clinical deterioration as a consequence of a defect in the urea cycle have considerably higher levels. In patients with intermittent signs and symptoms plasma ammonia may be normal when they are well. In addition, in some patients with ASL deficiency or in heterozygous females with OTC deficiency, plasma ammonia concentration may be normal, despite overt encephalopathy.

The concentration of the amino acids in the metabolic pathway immediately proximal to the enzyme defect will increase, and those beyond the block will decrease. Plasma alanine and glutamine accumulate in all disorders. Orotic acid and orotidine are excreted in the urine in defects distal to the formation of carbamoylphosphate (OTC, ASS, ASL and arginase deficiency).

Additional metabolic studies should include plasma amino acid analysis and urine organic acid analysis, as these may help to determine the site of the metabolic block in the urea cycle (◘ Table 20.1) as well as in the differentiation of hyperammonaemia from other causes (◘ Table 20.2). As urea cycle defects comprise the most devastating inborn errors of metabolism, and as initiation of the most appropriate therapy is of the utmost urgency, metabolic analysis should be done as the highest priority.

Plasma concentration of the transaminases ASAT and ALAT can be either normal or abnormal in urea cycle defects and liver function tests such as clotting studies are generally normal. Significant liver disease is most frequently seen in patients with ASL deficiency and with citrin deficiency. As mild hepatomegaly is common during acute metabolic derangements in urea cycle defects, a misdiagnosis of an infectious hepatitis is easily made. Imaging of the liver is not helpful in the diagnosis or immediate management of urea cycle defects.

Careful history taking, in combination with immediate laboratory studies, is needed to exclude other causes of hyperammonaemia (◘ Table 20.2). Metabolic disorders causing hyperammonaemia that can be relatively easily excluded by urgent metabolic studies are organic acidaemias (methylmalonic acidaemia, propionic acidaemia) and fatty acid oxidation defects. In these disorders glutamine is generally normal to low (▶ Chapter 19). In addition, routine laboratory studies may also help in the initial differential diagnosis with metabolic acidosis with increased anion gap and significant ketosis, suggestive of the

Table 20.2. Differential diagnosis of hyperammonaemia

Inherited disorders
- Urea cycle enzyme defects
  - Carbamoyl phosphate synthetase 1 deficiency
  - Ornithine transcarbamoylase deficiency
  - Argininosuccinate synthetase deficiency (citrullinaemia type 1)
  - Argininosuccinate lyase deficiency (argininosuccinic aciduria)
  - Arginase deficiency
  - *N*-Acetylglutamate synthetase deficiency
- Transport defects of urea cycle intermediates
  - Mitochondrial ornithine transporter (H-H-H syndrome)
  - Aspartate-glutamate shuttle (citrin) deficiency
  - Lysinuric protein intolerance
- Organic acidaemias
  - Propionic acidaemia
  - Methylmalonic acidaemia and other organic acidaemias
- Fatty acid oxidation disorders
  - Medium-chain acyl-CoA dehydrogenase deficiency
  - Systemic carnitine deficiency
  - Long-chain fatty acid oxidation defects and other related disorders
- Other inborn errors
  - Pyruvate carboxylase deficiency (neonatal form)
  - Ornithine aminotransferase deficiency (neonates/infants)
  - Hyperinsulinism-hyperammonaemia syndrome

Acquired disorders
- Transient hyperammonaemia of the newborn
- Any severe systemic illness particularly in neonates
- Herpes simplex
- neonates with systemic infection
- Liver failure
- Infection with urease-positive bacteria (with urinary tract stasis)
- Reye syndrome
- Valproate therapy
- Leukaemia therapy including therapy with asparaginase (rare)

Technical
- Inappropriate sample (e.g. capillary blood) or sample not immediately analysed

Anatomical variants
- Porto-systemic shunt

presence of an organic acidaemia as the cause of the hyperammonaemia, and the presence of hypoglycaemia with low to absent ketone bodies suggesting a fatty acid oxidation disorder or the hyperinsulinism-hyperammonaemia syndrome as the cause of the hyperammonaemia.

In summary the following investigations should be performed in patients with suspected hyperammonaemia:
- Repeated plasma ammonia
- Blood pH and blood gas analysis
- Electrolytes, lactate, urea, glucose, creatinine
- ASAT, ALAT, albumin, clotting tests
- Plasma amino acids
- Urinary orotic acid and organic acids
- Plasma free carnitine and acylcarnitines

Loading tests can be dangerous and should only be used in rare cases.

20.6 Treatment

20.6.1 Treatment in Acute Presentations

Emergency treatment for patients presenting with an acute metabolic crises with hyperammonaemia due to a (suspected) urea cycle disorder is aimed at:
1. Rapid removal of toxic NH_3 from body fluids.
2. Inhibition of production of NH_3.
3. Correction of any disturbance in electrolytes, acid-base state and prevention of dehydration or fluid overload.
4. If present, treatment of the intercurrent illness which triggered the acute metabolic compensation.

Emergency treatment should be initiated without delay in all patients with (progressive) encephalopathy and hyperammonaemia. Although there are no absolute levels of plasma ammonia that should lead to a full-scale emergency treatment, in any newly diagnosed patient ammonia concentrations >200 µmol/l should be followed by emergency management. As there are no high-level evidence based guidelines for the acute treatment of hyperammonaemia, guidelines are generally based on clinical experience and case histories or small series. As hyperammonaemia is a rare metabolic derangement which may rapidly cause irreversible neurological damage, treatment should preferably be coordinated by a metabolic centre with experience in urea cycle defects, and emergency referral of the patients is generally the best option.

1. Rapid Removal of Toxic NH_3 from Body Fluids

Dialysis

As the risk for irreversible cerebral damage is directly related to NH_3 concentration in plasma, immediate measures to decrease plasma levels of NH_3 are necessary in patients with high or rapidly rising concentrations. There are no evidence based data on the precise levels which should lead to the invasive procedure of extracorporeal detoxification, but levels >350 µmol/l in neonates or young infants should lead to preparation for extracorporeal detoxification, and if levels are >500 µmol/l this procedure should be started without delay.

Haemodialysis (HD) is superior to all other dialysing techniques such as haemofiltration or peritoneal dialysis, as it has a very high clearance of ammonia resulting in a reduction of > 50% within hours [17]. HD also results in a clearance of amino acids including glycine and glutamate, further enhancing its disposal of waste nitrogen [18]. One potential drawback of haemodialysis is that it efficiently removes nitrogen scavenging drugs as sodium benzoate and sodium phenylbutyrate [19] but its overall efficiency in removal of NH_3 overrides this potential side effect. However, continuous high-volume haemodialfiltrationor haemofiltration can be effectively used where HD is unavailable or judged to be impracticable due to an infant's size [20,21].

▪▪ Medication Providing Alternative Pathways for Nitrogen Excretion

Benzoate and phenylacetate (given orally as phenylbenzoate) can conjugate with the amino acids glycine and glutamine, respectively, and the products formed, hippurate and phenylacetylglutamine, can be excreted in the urine (◘ Fig. 20.2).

Full conjugation of benzoate with glycine results in an equimolar disposal of 1 mol nitrogen per mole benzoate (glycine contains one N atom), while full conjugation of 1 mol phenylacetate with 1 mol glutamine can result in the removal of 2 mol nitrogen (glutamine contains two N atoms).

No dose-finding studies have been performed, but based on biochemical rationale and experience the following guidelines can be given [22-24]:

- Sodium-benzoate: 250 mg/kg in 2 h i.v., followed by 250-500 mg/kg in 24 h
- Sodium-phenylacetate: 250 mg/kg in 2 h i.v., followed by 250-500 mg/kg in 24 h

The combination of sodium benzoate and sodium phenylacetate (10%/10% solution) is available for intravenous use (Ammonul®). If Ammonul® is not available, a combination of sodium benzoate i.v. and sodium phenylbutyrate (Ammonaps®) p.o. (250 mg/kg p.o., followed by 250-500 mg/kg in 24 h in three or four doses) may provide an alternative. However, due to decreased gastrointestinal motility, availability of oral medication may be significantly decreased.

High doses of sodium benzoate and sodium phenylacetate may result in adverse effects (vomiting, hyperglycaemia, hypokalaemia, convulsions) and in glutamine depletion, and dose adjustment may be necessary.

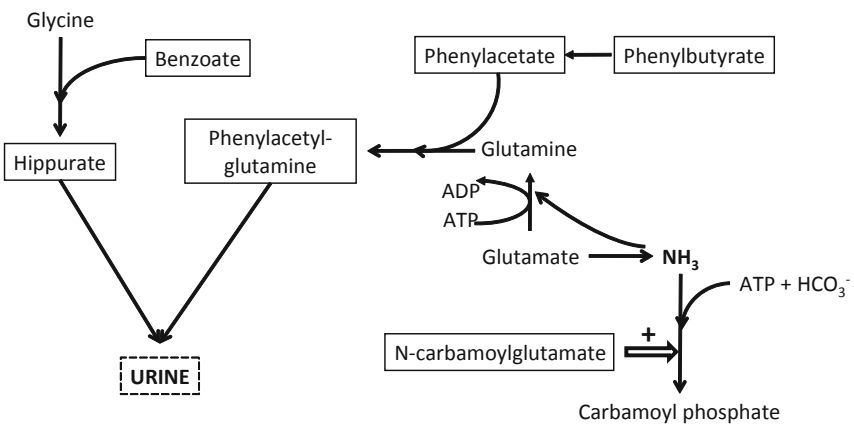
▪▪ Arginine and Citrulline

Arginine and citrulline can be used to promote the excretion of waste nitrogen. Supplementation of arginine in patients with ASS or ASL deficiency may result in removal of nitrogen in the form of citrulline and argininosuccinate, molecules that can be both excreted in the urine in large amounts. In addition, arginine deficiency is a common complication in urea cycle defects (◘ Table 20.1). Arginine should not be given in patients with (suspected) arginase deficiency.

The initial dose in patients with acute hyperammonaemia:

- Arginine hydrochloride 360 mg/kg in 2 h i.v., followed by a maintenance dose of 180-360 mg/kg per 24 h

In patients with OTC deficiency, arginine will not be effective for the promotion of excretion of waste nitrogen (◘ Fig. 20.1). Citrulline supplementation may result in the disposal of nitrogen by condensation with aspartate (1 nitrogen atom) and will also correct the arginine deficiency in OTC deficiency.



◘ **Fig. 20.2.** Mode of action of the nitrogen scavengers benzoate and phenylbutyrate, providing alternative pathways for ammonia disposal and of *N*-carbamoylglutamate in NAGS deficiency

- **2. Inhibition of Production of NH_3**

Caloric Supplementation

Immediate high-dose caloric supplementation by means of glucose i.v. is essential in order to stop protein catabolism and to promote anabolism. In general, glucose as a 10% or 20% solution is given and the dose is calculated to cover at least the full caloric need calculated for age and weight, mostly with a 10-20% surplus. For a full-term neonate this will be approximately 6 ml/kg per hour of a glucose 10% solution. Intravenous lipids (2-3 g/kg per day) and a continuous i.v. supply of direct-acting insulin may be added to prevent hyperglycaemia and to promote anabolism [23].

In addition, protein intake should be immediately stopped. This should, however, be limited to about 24 h after the start of treatment in order to prevent a protein catabolic state. Either enteric protein supplementation, at least by providing essential amino acids by means of special formulas, or, if enteric feeding is not feasible owing to the clinical condition of the patient, by amino acid mixtures i.v., is needed.

Carbamoyl Glutamate

In patients with NAGS deficiency and in some patients with CPS1 deficiency, the immediate supply of carbamoyl glutamate (Carbaglu®) as an artificial allosteric activator (instead of *N*-acetylglutamate; ◘ Fig. 20.1) of the enzyme CPS1, at a dose of 100 mg/kg orally per 24 h in three doses, may result in a rapid improvement of the metabolic and clinical condition [25, 26]. Therefore, if initial studies in a newborn with hyperammonaemia reveal normal orotic acid in urine and no accumulation of amino acids suggestive of a block downstream of CPS1, a trial with carbamoyl glutamate should immediately be started.

- **3. Correction of any disturbance in electrolytes, acid-base state and prevention of dehydration or fluid overload**

Sodium overload may be the result of the medication (sodium benzoate: 1 g contains approximately 7 mmol of Na^+; sodium-phenylbutyrate or acetate: 1 g contains approximately 6 mmol of Na^+), especially in newborns. Conversely, hypotonic solution may be responsible for cerebral oedema. In addition, hypokalaemia may be a side effect of sodium phenyl acetate. Frequent monitoring and corrections are necessary to prevent complications of rapid shifts in electrolyte concentrations. The mild respiratory alkalosis frequently observed in acute hyperammonaemia does not need any correction other than rapid removal of NH_3 from the circulation. Dehydration and fluid overloading should be prevented or corrected, as both may aggravate the clinical deterioration. Prevention of vomiting by medication may be necessary to prevent ongoing loss of fluids and electrolytes.

- **4. Treatment of the intercurrent illness that triggered the acute metabolic compensation**

Especially in older infants, children and adults, a metabolic decompensation is often triggered by an infectious disease. Therefore a full diagnostic work-up and aggressive treatment of infections is necessary.

20.6.2 Maintenance Therapy

Chronic management includes nutritional therapy, pharmacological therapy and essential aminoacid supplementation [24, 27, 28]. Management also aims to avoid metabolic decompensation during such at-risk periods as illness, fasting (e.g. before surgery) and exercise. More recently, liver and liver cell transplantation has been proposed as a treatment in urea cycle defects.

- **Nutritional Management**

A cardinal principle of management is the restriction of protein intake to minimise the flux of nitrogen through the urea cycle [24, 28, 29]. Protein intake should be limited to minimise hyperammonaemia, but must be sufficient to provide adequate essential amino acids to maintain cellular functioning and linear growth. The key to successful nutritional management is to maintain a positive nitrogen balance: protein synthesis must be greater than protein catabolism. However, protein and amino acid turnover are not static. Tissue protein is constantly being synthesised and catabolised, and ammonia detoxification will vary with growth, enzyme deficiency, activity level and the developmental and health status of the patient. The protein requirement at birth differs markedly from that at the end of the 1st year of life and is also different in the growing, pubertal child than in the full-grown adult. Titration of protein requirements during growth is empirical and varies from one child to another. In early infancy, patients may need >2 g/kg/day during phases of very rapid growth. The protein intake decreases to approximately 1.2-1.5 g/kg/day during preschool years and 0.8-1 g/kg/day in late childhood. The quantity of natural protein requirement may be less than 0.5 g/kg/day after puberty. Careful assessments are necessary to optimise the management. Somatic growth, well-being, and neurological status reflect long-term metabolic control. Some biological parameters reflect protein status: the haemoglobin level (several months), the plasma albumin level (the past 1-2 months), and the prealbumin level (the past 1-2 weeks). Plasma amino acids indicate protein status over a range of hours to days. It is

important to obtain fasting samples at similar times of the day to minimise variation [27] in the follow-up. Defects in the first part of the urea cycle, such as OTC and CPS1 deficiencies, tend to be more severe, because blockage occurs before any nitrogen can enter the cycle. The decreased nitrogen clearance in these disorders is not compensated for by excretion of metabolites such as citrulline.

Feeding difficulties are frequently seen in patients with urea cycle defects and may contribute to reduced caloric and protein intake and precipitation of catabolism. The underlying mechanisms are not fully defined, but may include side effects of pharmacotherapy, conditioning to adverse effects of protein loads, or neurological damage. Placement of a gastrostomy tube can contribute to dietary adherence and is recommended for very young patients. In addition to providing an enteral route for less palatable medications and special dietary formulas, a gastrostomy can be very helpful during periods of illness and refusal to feed. New surgical techniques have significantly decreased anaesthetic and technical complications of gastrostomy placement [30]. Oral feeding should be encouraged as much as possible to allow continued oral motor development and to reduce the risk of patients' developing oral aversion reactions. In patients with severe deficiencies and/or a 'brittle metabolic equilibrium', intravenous access can be needed frequently, and the possibility of immediate access can be life saving. For these patients, placement of an intravenous access device such as a Port-a-Cath® should be considered.

Routine vaccinations should be given, and additional preventive measures, such as respiratory syncytial virus prophylaxis, rotavirus and influenza vaccinations, should be considered. Temporary increases in pharmacotherapy should be initiated during periods that may precipitate a metabolic derangement due to increased protein catabolism.

Overzealous protein restriction is a common cause of metabolic instability, because this induces a shift to a negative nitrogen balance. Paradoxically high levels of ammonia in the morning and low levels of plasma branched-chain aminoacids are two of the most important biological signs of overzealous protein restriction.

Medical events such as viral illness, trauma and surgery, and also pregnancy and the puerperium, are situations that can induce a hyperammonaemic crisis. A sick-day diet regimen is required for such situations. This regimen will provide additional energy and decreased protein intake to avoid a negative nitrogen balance. For example, protein intake is decreased to 50% for 24-48 h, while nonprotein calories are increased by 25-50%.

In marked contrast, patients with citrin deficiency appear to benefit from a low-carbohydrate, high-protein diet, although it might be necessary to be cautious about the amount of protein if hepatic ASS activity is secondarily decreased [3].

▪ Pharmacological Therapy

Additional therapy is necessary is many patients. Sodium benzoate and sodium phenylbutyrate (Ammonaps®, or Buphenyl®), in doses up to 250 mg/kg/day, may prevent hyperammonaemia. *N*-Carbamoylglutamate (100-300 mg/kg/day) can be used in NAGS deficiency to replace the missing *N*-acetylglutamate and in patients with kinetic mutations in CPS1 [31]. Patients who respond may only require this treatment.

▪ Replacement of Deficient Nutrients and Essential AminoAcid Supplementation

In all patients with urea cycle defects (except for arginase deficiency), plasma arginine levels will be decreased due to deficient synthesis, and patients need to be supplemented with arginine (50-150 mg/kg/day). In OTC and CPS1 deficiencies, citrulline (50-150 mg/kg/day) may be used as a substrate for arginine synthesis.

In addition, both arginine and citrulline supplementation may lead to dispose of waste nitrogen (► Section 20.6.1).

In patients who need a significant restriction of natural protein, supplementation with special formulas containing essential amino acids and other nutrients may be essential for growth and development.

▪ Parameters to Assess Efficacy of Maintenance Therapy

Growth and weight should be followed meticulously, as these parameters will reflect the completeness of the diet. Regular monitoring of plasma levels of amino acids, with particular emphasis on essential amino acids, can be used to assess dietary completeness. Maintaining the concentrations of branched-chain amino acids, and in particular the concentration of leucine, within a near-normal range appears to be important, especially in patients receiving sodium phenylbutyrate treatment [24].

Plasma ammonia concentration should be kept within the normal range. A problem is that ammonia levels fluctuate and that levels measured during follow up in the hospital may not always reflect fluctuating ammonia levels at home. The value of home monitoring by means of the 'bedside' Ammonia Checker (e.g. BAC II®) for monitoring patients with urea cycle defects has not been established.

Plasma glutamine is probably the most important indicator for assessment of both chronic dietary and pharmacological control, and levels should preferably be

kept below 800 µmol/l [32], or, if this is not feasible, at least below 1000 µmol/l.

- **Emergency Protocol**

Even with vigilant dietary and pharmacological treatment, prompt intervention during a minor illness is often necessary to treat recurrent imminent episodes of metabolic decompensation with hyperammonaemia. The family, the primary care physician and the local emergency room must be educated regarding the necessity of prompt evaluation and treatment. They must be alerted to the child's disease, the potential for rapid deterioration, and the need for immediate attention and action. Most patients benefit from a written emergency protocol providing basic information about the disorder, necessary diagnostic investigations and guidelines for emergency treatment. Surgical procedures must be performed in a hospital with a full metabolic service. A 1-day surgery clinic is generally not recommended [33]. Preoperative fasting should be avoided, with glucose infusion being started preoperatively and continuing postoperatively until the child is eating and drinking well.

- **Supportive Treatment**

Many patients with urea cycle defects have neurological sequelae, and it is essential to provide an early developmental programme and specific treatments against seizures. Valproic acid is not recommended in these disorders [34]. Physiotherapy may enhance mobility and prevent medical complications, including respiratory problems. Raising a child with a chronic illness, with the continuous risk of severe metabolic derangement, can be extremely stressful for families. The psychological impact is often severe, with disturbances of employment activities, economic status and the family's relationship with the community. Families and patients need a multidisciplinary approach which includes the family, the dietitian, the local primary care team, psychosocial support and the metabolic centre [35].

- **Liver Transplantation and Liver Cell Transplantation**

A transfer of enzyme activity by liver transplantation or liver cell transplantation may be an appropriate strategy in severe urea cycle disorders. The 5-year survival rate for children after liver transplantation for metabolic disease has improved over the last decades and is now approximately 90% [36]. Liver transplantation is currently the only definitive cure for urea cycle defects. Between 1988 and 2004 a total of 113 patients underwent liver transplantation for a urea cycle defect in the USA [37]. Overall 5-year patient survival was excellent, at 86%. Similar results were described in the survey by Leonard and McKiernan, which included 59 patients [38]. Quality of life substantially improved in all surviving children, and discontinuation of the diet and reduction of hospital admissions were considered most important. As recurrent metabolic crises may result in further neurological damage, it is important to perform a liver transplantation early in those patients who are judged to be candidates for the procedure. Unfortunately, liver transplantation in neonates and small infants is technically challenging and still has a high complication rate.

Liver cell transplantation is much less invasive. Currently, reports of 10 patients who have received liver cell transplantation for the treatment of a urea cycle defect have been published. Safety and efficacy data from individual therapeutic attempts are encouraging. The key issue in human liver cell transplantation is the question of long-term efficacy [39]. Clinical applications of the use of stem cells are not yet established.

Liver transplantation has also been successfully used in the management of citrin deficiency, more often in adults with CTNL2, but also in those with NICCD [3].

20.7 Outcome

Despite major advances in both emergency and maintenance treatment for patients with urea cycle defects, the outcome in terms of survival and preservation of neurological functions is still far from satisfactory [40, 41]. Both the age at presentation and the enzyme deficiency involved correlate with outcome, neonatal presentation and both OTC deficiency in boys and ASS deficiency being associated with a high mortality and severe neurological sequelae [41, 42]. Early intervention aimed at rapid removal of NH_3 improves the outcome [43], but it remains to be seen whether neurological damage can be fully prevented. The value of newborn screening for urea cycle defects is debatable, as patients with the severe neonatal presentation will generally be severely ill by the time the results of the test are known. However, for milder variants early detection by newborn screening may result in a more favourable outcome [4].

20.8 Pregnancy

Successful pregnancies have been reported in patients with attenuated OTC and CPS1 deficiency. If carefully managed during and after pregnancy, an uncomplicated outcome for both mother and child can be achieved [44-46] However, if these conditions remain unrecognised or untreated, the puerperium appears to be the

period with the highest risk of life-threatening hyperammonaemia in the mother [45, 46].

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Disorders of Sulfur Amino Acid Metabolism

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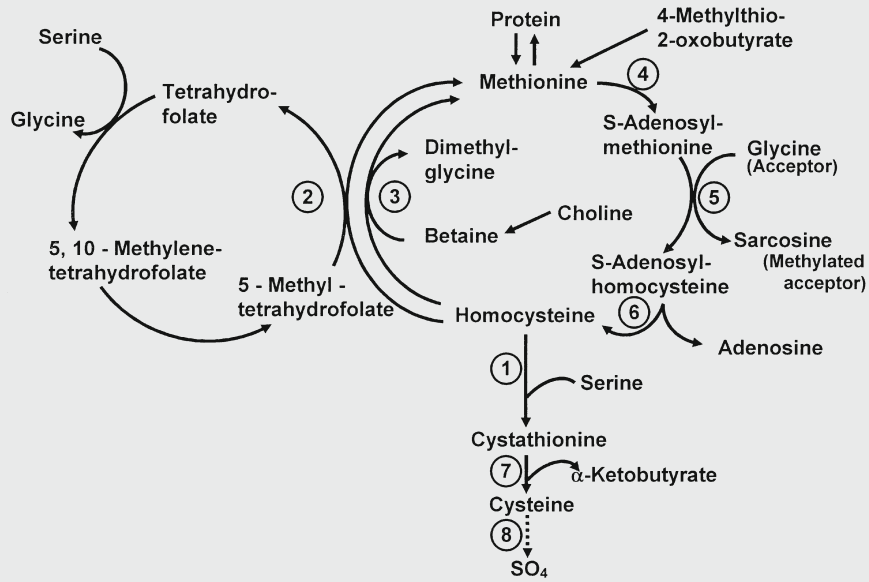
Metabolism of the Sulfur-Containing Amino Acids

Methionine, homocysteine and cysteine are linked by the methylation cycle (◘ Fig. 21.1, left part) and the *trans*-sulfuration pathway (◘ Fig. 21.1, right part). Conversion of methionine into homocysteine proceeds via methionine *S*-adenosyltransferase (enzyme 4). This yields *S*-adenosylmethionine, the methyl-group donor in a wide range of transmethylation reactions, a quantitatively important one of which is glycine *N*-methyltransferase (enzyme 5). These reactions also produce *S*-adenosylhomocysteine, which is cleaved to adenosine and homocysteine by *S*-adenosylhomocysteine hydrolase (enzyme 6). Depending on a number of factors, about 50% of available homocysteine is recycled into methionine. This involves methyl transfer from either 5-methyl-tetrahydrofolate (THF), catalysed by cobalamin-requiring 5-methyl THF-homocysteine methyltransferase (enzyme 2), or betaine, catalysed by betaine-homocysteine methyltransferase (enzyme 3). Homocysteine can also be condensed with serine to form cystathionine via a reaction catalysed by pyridoxal-phosphate-requiring cystathionine β-synthase (enzyme 1). Cystathionine is cleaved to cysteine and α-ketobutyrate by another pyridoxal-phosphate-dependent enzyme, γ-cystathionase (enzyme 7). The last step of the *trans*-sulfuration pathway converts sulfite to sulfate and is catalysed by sulfite oxidase (enzyme 8), which requires a molybdenum cofactor.



◘ **Fig. 21.1.** Metabolism of the sulfur-containing amino acids. **1**, Cystathionine β-synthase; **2**, 5-methyltetrahydrofolate-homocysteine methyltransferase; **3**, betaine-homocysteinemethyltransferase; **4**, methionine *S*-adenosyltransferase; **5**, glycine *N*-methyltransferase; **6**, *S*-adenosylhomocysteine hydrolase; **7**, γ-cystathionase; **8**, sulfite oxidase

Several inherited defects are known in the conversion of the sulfur-containing amino acid methionine to cysteine and the ultimate oxidation of cysteine to inorganic sulfate (◘ Fig. 21.1). Cystathionine β-synthase (CBS) deficiency is the most important. It is associated with severe abnormalities of four organs or organ systems: the eye (dislocation of the lens), the skeleton (dolichostenomelia and arachnodactyly), the vascular system (thromboembolism), and the central nervous system (mental retardation, cerebrovascular accidents). A low-methionine, high-cystine diet, pyridoxine, folate and betaine in various combinations, and antithrombotic treatment may halt the otherwise unfavourable course of the disease. Methionine *S*-adenosyltransferase deficiency and γ-cystathionase deficiency usually do not require treatment. Isolated sulfite oxidase deficiency leads (in its severe form) to refractory convulsions, lens dislocation and early death. No effective treatment exists. Combined deficiency of sulfite oxidase and xanthine oxidase is discussed in Chapter 36. Deficiencies of glycine *N*-methyltransferase and *S*-adenosylhomocysteine hydrolase have been described in a few patients.

21.1 Homocystinuria Due to Cystathionine β-Synthase Deficiency

21.1.1 Clinical Presentation

The eye, skeleton, central nervous system, and vascular system are all involved in the typical presentation. The patient is normal at birth and, if not treated, progressively develops the full clinical picture, which may in extreme cases appear early in life.

- **Eye**

Dislocation of the ocular lens (ectopialentis), myopia and glaucoma are frequent, severe and characteristic complications. Retinal detachment and degeneration, optical atrophy and cataracts may eventually appear. Myopia may precede lens dislocation, and in this case worsens afterwards. Ectopia lentis is detected in most untreated patients from 5 to 10 years of age and in nearly all untreated patients by the end of the 4th decade and is often the clue to diagnosis. The dislocation is generally downward, whereas it is usually upward in Marfan syndrome, a phenocopy of homocystinuria caused by mutations of the fibrillin-1 gene. Once ectopia lentis has occurred, a peculiar trembling of the iris (iridodonesis) following eye or head movement may be evident.

- **Skeleton**

Osteoporosis is almost invariably detected, at least after childhood. Frequent consequences are scoliosis and a tendency towards pathological fractures and vertebral collapse. As in Marfan syndrome, homocystinuric patients tend to be tall, with thinning and elongation (dolichostenomelia) of long bones near puberty, enlarged metaphyses and epiphyses, especially at the knees, and arachnodactyly, which is present in about half the patients. Other bone deformities include genu valgum with knobbly knees, pescavus and pectus carinatum or excavatum. Restricted joint mobility, particularly at the extremities, contrasts with the joint laxity observed in Marfan syndrome. Abnormal X-ray findings include biconcavity and flattening of the intervertebral discs, growth arrest lines in the distal tibia, metaphyseal spicules in the hands and feet, enlarged carpal bones, retarded lunate development and shortening of the fourth metacarpal.

- **Central Nervous System**

Developmental delay and mental retardation affect about 60% of patients to a variable degree. Seizures, electroencephalogram abnormalities and psychiatric disturbances have also been reported in approximately half of all recorded cases. Focal neurological signs may be a consequence of cerebrovascular accidents.

- **Vascular System**

Thromboembolic complications, occurring in arteries and veins of all parts of the body, constitute the major cause of morbidity and mortality. The prognosis is influenced by the site and extent of the vascular occlusion. Thrombophlebitis and pulmonary embolism are the most common vascular accidents. Thrombosis of large and medium arteries, particularly carotid and renal arteries, is a frequent cause of death. Ischaemic heart disease is a less prominent feature of homocystinuria. Association with other genotypes linked to increased risk of vascular disease, such as the factor V Leiden R506Q mutation or the 677C→T mutation of the *MTHFR* gene, were reported to increase the risk of thrombosis in homocystinuric patients [1].

- **Other Features**

Spontaneous pneumothorax and pancreatitis were reported to be rare findings in homocystinuric patients [2].

- **Clinical Variability and Natural History**

The spectrum of clinical abnormalities is wide, and mild cases may only be recognised by late complications, such as thromboembolic accidents. Time-to-event curves based on detailed information on 629 patients were calculated by Mudd et al. [3] for the main clinical manifestations and mortality. Each abnormality occurred significantly earlier and at a higher rate in untreated pyridoxine-nonresponsive individuals than in untreated pyridoxine-responsive ones. The risk of thromboembolic accidents in patients undergoing surgery was relatively small, complications, six of which were lethal, being recorded in only 25 patients following 586 operations.

An Italian multicentre survey [2] revealed a strong correspondence of ectopia lentis, mental retardation, seizures, dolichostenomelia and thrombotic accidents among affected sib-pairs, supporting a prominent role of genetic factors in determining the phenotype. Nevertheless, rare cases of intrafamilial variability have been reported. Probably, both early diagnosis and strict compliance to treatment will change the natural history of cardiovascular and mental manifestations even in pyridoxine-nonresponsive individuals.

In a paper revisiting the natural history [4] it is pointed out that homozygosity for mild mutations such as c.833T>C (p.I278T9), calculated on the basis of the mutant allele frequency, might be more frequent than previously indicated. However, the resulting phenotype would be very mild or would appear late in adulthood

with clinical features, particularly cardiovascular manifestations, and such subjects might easily be misdiagnosed as having a common multifactorial disorder. This revisit suggests that care is needed in use of the previously published time-to-event curves [3].

- **Outcome of Pregnancies**

Pyridoxine-responsive women are able to sustain pregnancies without a significant risk of malformations in the offspring. There is much less experience of outcome of pregnancies in nonresponsive women. Details of 15 pregnancies in 11 women, 5 of whom were pyridoxin enonresponsive, were reported [5], and complications such as pre-eclampsia, superficial venous thrombosis and first-trimester spontaneous abortion were observed in two pregnancies. Ten pregnancies produced normal live-born infants, while one offspring had multiple congenital anomalies and another had Beckwith-Wiedemann syndrome. No relationship could be established between the severity of biochemical abnormalities during pregnancy and either pregnancy complications or offspring outcome. The results of this study suggest that pregnancy complications and offspring abnormalities are infrequent events. Nevertheless, careful monitoring of these pregnancies is mandatory.

21.1.2 Metabolic Derangement

Cystathionine β-synthase (CBS) activity can be found in many tissues, including liver, brain, pancreas and cultured fibroblasts. In addition to the coenzyme pyridoxal phosphate, CBS also binds two other ligands, the activator *S*-adenosylmethionine and a haem moiety of unclear function. In vivo responsiveness to pharmacological doses of pyridoxine, present in approximately 50% of homocystinuric patients, is generally associated with the presence of a small amount of residual enzymatic activity, although exceptions to this rule are known [6].

Deficiency of CBS leads to tissue accumulation of methionine and homocysteine and of their *S*-adenosyl derivatives, with lack of cystathionine and low levels of cysteine. The -SH group of homocysteine readily reacts with the -SH group of a second homocysteine molecule or of other molecules, leading to the formation of a number of disulfide compounds, such as homocystine, homocysteine-cysteine mixed disulfide or protein-bound homocysteine.

The pathophysiology of CBS deficiency has not yet been completely elucidated, but accumulation of homocysteine probably plays a major role in determining some of the most relevant clinical manifestations, including generalised vascular damage and thromboembolic complications. Thromboembolism has been suggested to be the end-point of homocysteine-induced abnormalities of platelets, endothelial cells and coagulation factors. There is accumulating evidence of a major role of the thioester homocysteine-thiolactone (Hcy-thiolactone) in the pathophysiology of atherothrombotic events in severe hyper-homocysteinaemic conditions whether or not these are due to CBS deficiency [7]. Hcy-thiolactone is generated during protein synthesis when homocysteine is erroneously selected in place of methionine by the specific Met tRNA-synthase. The resulting thioester causes *N*-homocysteinylation of lysine residues of proteins such as fibrinogen, albumin and low- and high-density lipoproteins. This post-translational modification would alter protein functioning, trigger an auto-immune response and induce cell damage and death.

Among other deleterious effects homocysteine may cause abnormal cross-linking of collagen, leading to abnormalities of the skin, joints and skeleton in patients. This mechanism seems unlikely to cause damage to the non collagenous zonular fibres of the lens which is more likely to be due to disturbed fibrillin structure.

Knowledge of the pathophysiology of CBS deficiency might be expected to be increased by the development of genetically modified animal models. Unfortunately, the resulting phenotype of the CBS knockout mouse is rapidly lethal [8]. Additional CBS genetically modified mouse models have been developed, but the resulting phenotypes, even after crossing with the CBS knockout strain, are very different from those observed in humans.

21.1.3 Genetics

Homocystinuria due to CBS deficiency is inherited as an autosomal recessive trait. Clinical and biochemical variations, such as pyridoxine responsiveness, are also genetically determined and related to specific mutations.

The worldwide frequency of homocystinuria has been reported to be 1 in 344,000, while that in Ireland is much higher, at 1 in 65,000. However, the results of mass screening using either molecular genetic tests or a biochemical test (see below) might disclose a higher incidence of this disorder, as shown in Denmark [4].

The *CBS* gene is located on chromosome 21 (21q22.3). The structure, function and regulation of CBS and location of homocystinuria-causing mutations have been investigated in depth [9]. Molecular studies on CBS patients have led to the characterisation of more than 150 mutations, most of which are private (website set up by Jan P. Kraus, Viktor Kozich and MiroslavJanosik: http://

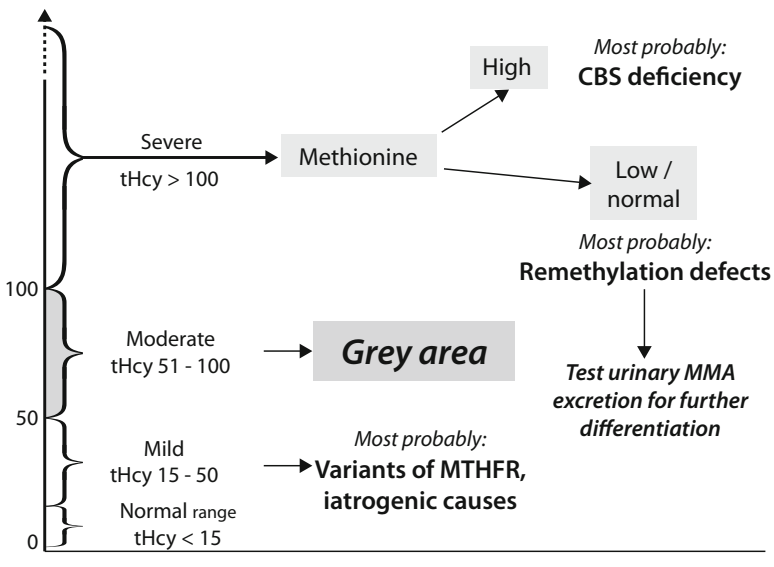
www.uchsc.edu/cbs/cbsdata/cbsmain.htm; website at GeneCards® http://www.genecards.org/cgi-bin/carddisp.pl?gene=CBS&search=CBS). Only a few mutations appear to be of epidemiological relevance: p.I278T, which is found in some 25% of homocystinuric alleles, and p.A114V are both associated with a pyridoxine-responsive form of the disease; p.G307S is mostly found in CBS patients of Irish origin and is not linked to response to pyridoxine. Compound heterozygotes show a variable response to pyridoxine, although the presence of the I278T mutation seems to confer pyridoxine responsiveness even in compound heterozygotes.

In at least 5% of caucasian alleles, exon 8 displays both a 68-bp duplication of the intron-exon junction and the I278T mutation [10], but a peculiar nucleotidic structure generated by the 68-bp duplication allows rescue of the wild-type sequence, so preserving the protein function [11].

21.1.4 Diagnostic Tests

The initial diagnosis is best achieved by quantitative amino acid analysis of plasma, which must be immediately processed to prevent loss of disulfide amino acids by binding to protein. Increased levels of methionine, homocystine and cysteine-homocysteine disulfide, low cystine and no increase of cystathionine, make up a cluster that is typical of CBS deficiency. Exceptionally, some pyridoxine-responsive patients are extremely sensitive to very small supplements of pyridoxine such as are contained in multivitamin tablets, so that false-negative results may be obtained. Determination of plasma total homocysteine, after treatment of the plasma sample with reducing agents, is useful for both preliminary diagnosis and monitoring of treatment. The determination of total plasma homocysteine and the various methods available have been comprehensively reviewed [12]. Normal plasma total homoyst(e)ine values are less than 15 µmol/l, whereas most untreated CBS patients exhibit levels above 200 µmol/l. Hyperhomocyst(e)inaemia also occurs in remethylation defects, owing to 5,10-methylene-tetrahydrofolate reductase deficiency and 5-methyl-THF-homocysteine-methyltransferase deficiency, whether isolated or due to defects in cytosolic cobalamin metabolism (▶ Chapter 28). These disorders can mostly be distinguished from CBS deficiency by the very low to normal plasma methionine level. It must be noted that, in CBS deficiency, methionine concentrations tend to decrease with age and may even be normal in some older patients. One factor in this decrease may be folate depletion and a consequent reduced capacity for remethylation. A simplified approach to the differential diagnosis of conditions causing hyperhomocyst(e)inaemia is shown in ◘ Fig. 21.2. Careful evaluation is required when homocyst(e)ine plasma levels fall in the grey area (50-100 µmol/l). *For these values* different causes should be considered, including very mild forms of CBS deficiency.

It needs to be borne in mind that a wide range of non-genetic causes of hyperhomocyst(e)inaemia are known, including end-stage renal disease and administration of drugs such as methotrexate, trimethoprin, phenytoin, sulfzalazine and niacin.



◘ **Fig. 21.2.** Differential diagnosis of conditions causing hyperhomocysteinaemia

Definitive diagnosis requires demonstration of greatly reduced CBS activity, usually assayed in cultured skin fibroblasts though this is also possible in phytohaemagglutinin-stimulated lymphocytes and liver biopsies. Exceptional patients may have significant residual activity of CBS in fibroblast extracts but still show the typical abnormalities of the disease.

The molecular diagnosis of CBS now provides a powerful additional approach to the diagnosis, though the high proportion of private mutations identified so far may represent a limit for this methodology or, at least, make it an expensive approach. Systematic screening for mutations of the entire coding region of the *CBS* gene is a prerequisite for reliable establishment of genotype/phenotype correlation, not least because a double mutational event has been observed on a single allele in some patients.

In many countries, newborn mass screening programmes based on detection of hypermethioninaemia have been implemented. A reduced cut-off value for methionine of 67 μmol/l was proposed to decrease the high rate of false-negative results previously reported in pyridoxine-responsive patients [13].

A reliable newborn screening has been developed by measuring homocysteine levels in dried blood spots on Guthrie cards by sensitive high-performance liquid chromatography-tandem mass spectrometry [14].

The cyanide-nitroprusside rapid test on urine, the so-called Brand test, is still used, but one should be aware of its weak sensitivity and specificity.

▪ Prenatal Diagnosis

Prenatal diagnosis of homocystinuria has been performed in at-risk pregnancies by assaying CBS in extracts of cultured amniocytes [15]. CBS activity is very low in uncultured chorionic villi from control subjects and can only be measured after culturing. In the families where mutation(s) is known, direct analysis of the *CBS* gene allows rapid prenatal diagnosis and in other cases DNA linkage analysis to the CBS locus may have diagnostic value.

▪ Heterozygotes

On a group basis, differences between obligate heterozygotes and control subjects have been clearly demonstrated using either measurements of homocyst(e)ine in plasma after methionine loading or assay of CBS in liver biopsies, cultured skin fibroblasts or phytohaemagglutinin-stimulated lymphocytes. However, overlap of values obtained in a considerable number of obligate heterozygotes with those at the lower end of the control range limits the value of these two approaches for heterozygote testing in individual subjects.

Molecular analysis of established mutations allows heterozygote detection in individual families, and the most common CBS mutations might be considered in population screening for CBS heterozygotes. Fibroblast CBS activity compatible with a heterozygosity was found in a significant number of vascular disease patients with hyperhomocyst(e)inaemia. However, molecular genetic studies failed to demonstrate a causative role of CBS heterozygosity in patients affected by premature vascular disease [16].

21.1.5 Treatment and Prognosis

The aim of treatment is to reduce plasma total homocyst(e)ine levels to as close to normal as possible while maintaining normal growth rate. Plasma cystine should be kept within the normal range (67±20 μmol/l) and should be supplemented if necessary (up to 200 mg/kg/day). Homocysteine levels can be lowered in a number of ways, and the best approach or combination for the individual patient will depend on the nature of the defect and social factors.

About half of all patients with CBS deficiency respond, often only partially, to large oral doses of pyridoxine. In about 10% of the patients who respond fully, fasting plasma total homocysteine, methionine and cystine become normal following a period of up to a few weeks of daily administration of between a few milligrams and 1000 mg of pyridoxine. Response to the vitamin is also influenced by folate depletion, which may be due to pyridoxine administration itself. Therefore, folic acid (5-10 mg/day) should be added to the treatment in combination with vitamin B_{12} (1mg/single injection/month) if a reduced serum level is demonstrated. An approach to assessment of pyridoxine responsiveness is to begin with 100 mg/day and, if necessary, progressively increase this to 500-1000 mg/day with monitoring of plasma levels of methionine and total homocysteine every other day.

Since doses higher than 1000 mg/day have been associated with sensory neuropathy, pyridoxine should be kept at the lowest dose able to achieve adequate metabolic control. In particular, doses greater than 250 mg/day should be avoided in newborns and young infants. In patients who do not respond to pyridoxine, a low-methionine/high-cystine diet must be introduced and must be continued throughout life.

A less strict low-methionine diet may also be necessary to achieve adequate control in pyridoxine-responsive patients. Synthetic methionine-free amino acid mixtures are commercially available and are especially useful for infants. The requirement for methionine is met by small amounts of infant formula. Supplements of essential fatty

acids and carbohydrates are also required if not present in the methionine-free amino acid mixture. After infancy, foods containing proteins low in methionine can be introduced, including gelatin and pulses such as lentils and soybeans. However, it should be noted that soya-modified formulas are usually enriched with methionine. In addition to pyridoxine, folate and, possibly vitamin B_{12}, the usual vitamin and mineral supplements are recommended.

Betaine, given orally at a maximum dose of 150 mg/kg/day (6-9 g maximum in adults) is another important homocysteine-lowering agent, which is especially useful when compliance with the diet is unsatisfactory. For older children and adults 6-9 g betaine are given daily, divided into three doses. Betaine remethylates homocysteine, often leading to very high methionine concentrations but with no apparent influence on the pathophysiology of the disease. However, unexplained cerebral oedema has been described in a few children receiving betaine therapy [17].

Vitamin C supplementation (1 g/day) has been shown to ameliorate endothelial dysfunction in CBS patients, suggesting its possible value in reducing the long-term risk of atherothrombotic complications. The value of long-term treatment with antithrombotic agents such as dipyridamole (100 mg four times per day) either alone or combined with aspirin (100 mg/day) remains to be proven. In the meantime, the need of such treatment should be assessed on an individual basis.

Whatever the combination of regimens employed, achievement of virtually normal total homocysteine levels is very difficult in most patients. This notwithstanding, prevention of the severe clinical abnormalities associated with this disorder requires lifelong treatment, and considerable impact on outcome has been achieved in patients for whom adequate treatment was judged as removal of free-disulfide homocystine from plasma. The results of the international survey provide a firm baseline for the evaluation of past and future therapeutic regimens [3], although the possible ascertainment bias in the time-event curves describing the natural history of untreated CBS deficiency suggests caution in their use [4]. When the low-methionine diet was started in the newborn period, mental retardation was prevented, the start and progression of lens dislocation were delayed and the incidence of seizures decreased. When late-diagnosed, responsive subjects received pyridoxine treatment, the first thromboembolic episode occurred later. In fact, a normal IQ was reported in teenage pyridoxine-nonresponsive CBS individuals with good compliance with treatment since birth [18]. Also treatment regimens aimed at lowering plasma homocysteine significantly reduce cardiovascular risk in homocystinuric patients despite imperfect biochemical control [19].

Finally, the success of treatment clearly depends on early diagnosis and treatment, providing a case for mass newborn screening.

A promising theurapeutic strategy is based on chaperone molecules which are able to improve the misfolding of a mutated enzyme. Recently, an increase in enzyme activity for a large number of CBS mutant enzymes has been obtained by using ligands and chemical chaperones [20, 21] or inducers of Hsp70 protein, which acts as a natural chaperone [22].

21.2 Methionine S-Adenosyltransferase Deficiency

21.2.1 Clinical Presentation

More than 60 patients with methionine *S*-adenosyltransferase (MAT) deficiency have now been described, many detected by newborn screening, and the great majority have so far been symptom free, suggesting a benign disorder [23]. Population screening [24] suggests an incidence as high as 1 in 28,163. However, neurological abnormalities and demyelination of the brain attributed to deficient formation of *S*-adenosylmethionine have been observed in a few patients, possibly linked to the severity of the enzyme deficiency.

21.2.2 Metabolic Derangement

This disorder is characterised by a deficiency of the hepatic form of the enzyme MAT I/III (but not the extrahepatic form, MAT II), leading to elevated methionine concentrations in tissues and physiological fluids. The degree of hypermethioninaemia seems to be associated with the type of mutation. The product of this enzyme reaction, *S*-adenosylmethionine, appears not to be deficient in most cases. Alternative metabolism of methionine seems to occur above a threshold plasma methionine concentration of about 300 μM, resulting in the formation of the transamination product 4-methylthio-3-oxobutyrate and dimethyl sulfide, the latter resulting in a distinctive odour of the breath.

21.2.3 Genetics

Three forms of MAT are known: MAT-I, -II, and -III. MAT-I and -III are encoded by the same gene, *MAT1A*, and correspond to tetrameric and dimeric forms of a single α1-subunit, respectively. MAT-II is encoded by a separate gene, mainly expressed in fetal liver and in

kidney, brain, testis and lymphocytes. Mutations of the *MAT1A* gene account for both autosomal recessive [25] and autosomal dominant hypermethioninaemia [26]. The rarer autosomal dominant form is caused by a mutation on a single allele with a dominant-negative effect.

21.2.4 Diagnostic Tests

High methionine in plasma and urine, detected by the usual chromatographic methods, without increased homocyst(e)ine of the degree seen in CBS deficiency and associated with no increase of *S*-adenosylmethionine, is suggestive of this defect, but several other causes of hypermethioninaemia are possible and must be excluded. Careful interpretation of results is needed, since elevated plasma total homocysteine of up to 59 μmol/l has been reported [27].

21.2.5 Treatment and Prognosis

Treatment is generally not indicated but, in patients with evidence of demyelination, administration of *S*-adenosylmethionine corrects deficiency of this compound. If the postulated association between specific mutations leading to a severe enzyme deficiency holds true [28, 29], treatment with *S*-adenosylmethionine may be advisable in such cases.

Four pregnancies in a woman with severe MAT I/III deficiency resulted in the birth of three normal children, with fetal arrest at 10-11 weeks in the other. Four women with mild hypermethioninaemia due to heterozygosity for the dominant R264H mutant allele gave birth to a total of 16 normal children, with just one recorded miscarriage [30].

21.3 Glycine N-Methyltransferase Deficiency

21.3.1 Clinical Presentation

Persistent isolated hypermethioninaemia associated with a history of persistent elevated plasma transaminases, and mild hepatomegaly were found in two siblings documented from the ages of 1 year and 5 years [31] and in an unrelated boy followed up from 2 years of age [32].

21.3.2 Metabolic Derangement

The constellation of high methionine, elevated *S*-adenosylmethionine in plasma without elevated *S*-adenosylhomocysteine and sarcosine provides strong circumstantial evidence of deficiency of glycine *N*-methyltransferase (GMT). No direct demonstration of deficiency of this liver enzyme has been possible, but the finding of mutations in the *GMT* gene, which revealed reduced enzyme activity in expression studies [32, 33], strongly supports GMT deficiency as a cause of these metabolic changes.

21.3.3 Genetics

The finding of compound heterozygosity for mutations in the *GMT* gene also occurring in either parent confirm autosomal recessive inheritance of this defect [33].

21.3.4 Diagnostic Tests

Differentiation between this defect and other forms of isolated hypermethioninaemia is possible by measurement of *S*-adenosylmethionine and *S*-adenosylhomocysteine in plasma. *S*-Adenosylmethionine levels are approximately 10- to 30-fold higher than the upper limit of controls in GMT deficiency, with normal levels in methionine adenosyltransferase deficiency. Sarcosine in plasma, *S*-adenosylhomocysteine and total homocysteine are not elevated.

21.3.5 Treatment and Prognosis

It has been speculated that treatment with a low-methionine diet supplemented with cystine might be beneficial, although it is questionable whether treatment is needed. This opinion may have to be modified, since phenotypic changes have been observed in the knockout mouse [34].

21.4 S-Adenosylhomocysteine Hydrolase Deficiency

21.4.1 Clinical Presentation

The first patient with this disorder [35] showed severely delayed psychomotor development and severe myopathy. When the condition was diagnosed the patient was 12.7 months old: development had ceased, and MRI of the brain showed delayed myelination and atrophy of white matter. Four further patients have been reported [24]. The two brothers of the index patient and an adult followed up from an early age showed a similar clinical picture to the first case, and one patient died at the age of 4 months from respiratory distress and liver failure.

21.4.2 Metabolic Derangement

Deficiency of this enzyme has been proven and leads to a block in the degradation and accumulation of *S*-adenosylhomocysteine and also to increased levels of *S*-adenosylmethionine and methionine. Elevated levels of guanidinoacetate and low levels of phosphatidylcholine and choline are compatible with inhibition of the respective transmethylases by *S*-adenosylhomocysteine. A number of other abnormalities, such as slightly elevated total homocysteine, betaine, dimethylglycine and cystathionine, remain unclear.

21.4.3 Genetics

An autosomal recessive inheritance is indicated, and sequencing of the *S*-adenosylhomocysteine hydrolase gene has revealed the presence of a maternally derived nonsense mutation and a missense mutation of paternal origin.

21.4.4 Diagnostic Tests

Differentiation between this and the two other forms of hypermethioninaemia described here can be achieved by measurement of *S*-adenosylhomocysteine, *S*-adenosylmethionine and sarcosine in plasma. Each of these is elevated, with approximately 100- and 30-fold elevations of *S*-adenosylhomocysteine and *S*-adenosylmethionine, respectively. Total homocysteine is normal or nearly normal, and hypermethioninaemia appears to be an inconsistent finding.

21.4.5 Treatment and Prognosis

Treatment in the form of severe restriction of methionine intake and administration of phosphatidylcholine in the form of egg yolk has been attempted. This resulted in lowering of plasma metabolites and clinical improvement, but the long-term outcome remains unknown.

21.5 γ-Cystathionase Deficiency

21.5.1 Clinical Presentation

This is considered to be a benign disorder. Subjects detected without ascertainment bias are mainly asymptomatic; subjects with mental retardation have had healthy siblings with the same defect or without the defect but showing the same symptoms. A recent study relating mutation studies to clinical presentation substantiates this view [36]. Also, it is noteworthy that knockout mice require dietary cysteine to protect them against acute lethal myopathy and oxidative injury [37].

21.5.2 Metabolic Derangement

Deficiency of the pyridoxal-phosphate-requiring γ-cystathionase leads to tissue accumulation of cystathionine. Increased plasma concentrations and markedly increased excretion of cystathionine occur, and *N*-acetylcystathionine is also excreted.

21.5.3 Genetics

Inheritance is autosomal recessive. The cystathionase gene was cloned [38], and mutant alleles in individuals with this condition have been reported [39].

21.5.4 Diagnostic Tests

High urinary excretion of cystathionine without homocystine and with normal plasma methionine points to this defect. Moderately elevated total homocysteine is an inconsistent finding [36]. Transient cystathioninuria in newborns is due to known secondary causes, such as vitamin-B_6 deficiency, generalised liver disease, thyrotoxicosis and neural tumours. Milder increases of plasma and urine levels of cystathionine can also occur in the remethylation defects due to overproduction of this metabolite. While γ-cystathionase activity is certainly expressed in cultured skin fibroblasts, the level of activity is probably too small to allow reliable measurement by specific enzyme assay [40].

21.5.5 Treatment and Prognosis

Most subjects respond to administration of about 100 mg of pyridoxine daily, though as this is a benign disorder it remains debatable whether treatment is needed.

21.6 Isolated Sulfite Oxidase Deficiency

21.6.1 Clinical Presentation

As recently reviewed [41], characteristic findings in the severe form of this enzyme deficiency, whether isolated (approximately 20 patients reported) or due to molyb-

denum cofactor deficiency (more than 50 described, ►Chapter 36), are early refractory convulsions, severe psychomotor retardation, failure to thrive, microcephaly, hypotonia passing into hypertonia, lens dislocation and early death [42]. A milder presentation has also been reported.

21.6.2 Metabolic Derangement

Sulfite oxidase catalyses the last step in the oxidation of the sulfur atom of cysteine into inorganic sulfate (◘ Fig. 21.1). Its deficiency results in accumulation of the suspected toxic compound sulfite together with its detoxification products, *S*-sulfocysteine and thiosulfate, with reduced formation of sulfate.

21.6.3 Genetics

This autosomal recessive disease has been explained at the molecular level by cloning of the gene and characterisation of mutations in several patients affected by the isolated sulfite oxidase deficiency [43, 44].

21.6.4 Diagnostic Tests

Increased sulfite can be detected with urine test strips, but samples must be fresh and, in one case, no increased sulfite with normal sulfate excretion was reported. *S*-Sulfocysteine is a stable, ninhydrin-positive diagnostic parameter that can be detected by electrophoresis or chromatography and can be quantified in urine and plasma by classic ion-exchange techniques. Reduced total homocysteine can be a useful pointer to the diagnosis [45].

Cystine levels are always very low. Thiosulfate can also be searched for by thin-layer chromatography. The absence of xanthinuria and the normal blood levels and urinary excretion of uric acid distinguish the isolated deficiency from the molybdenum-cofactor defect (►Chapter 36). Sulfite oxidase activity can be determined in cultured skin fibroblasts.

21.6.5 Treatment and Prognosis

Attempts at treatment have been mainly unsuccessful, although evidence of clinical improvement in two mildly affected patients on a low-cystine and low-methionine diet has been reported [46]. Attempts to remove sulfite by binding to penicillamine were unsuccessful [47].

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Disorders of Ornithine Metabolism

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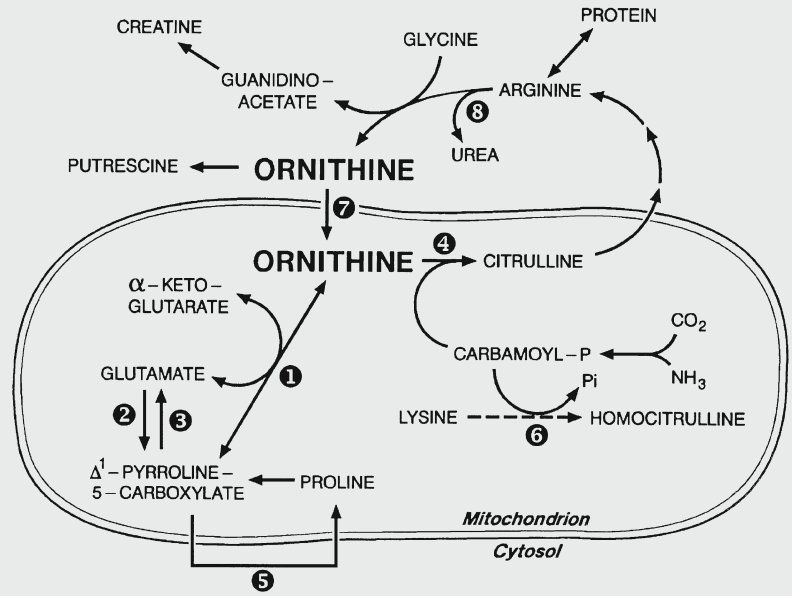
Ornithine Metabolism

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 Δ^1-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. 22.1). Since both OAT and ornithine transcarbamoylase (OCT) 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), a transporter in the inner mitochondrial membrane.

P5C, the product or precursor of the OAT reaction, is also a key intermediate in proline and glutamate metabolism. P5C synthase, 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. Two forms of P5C reductase, P5CR1 and P5CR2, are encoded by separate genes, and catalyse the conversion of P5C to proline.



◘ **Fig. 22.1.** Ornithine metabolic pathways. *Pi*, inorganic phosphate. **1**, Ornithine-δ-aminotransferase (OAT); **2**, Δ^1-pyrroline-5-carboxylate synthase (P5CS); **3**, Δ^1-pyrroline-5-carboxylate dehydrogenase; **4**, ornithine transcarbamoylase (OTC); **5**, Δ^1-pyrroline-5-carboxylate reductase; **6**, lysine transcarbamoylase (the step indicated by the *broken line* is not well defined); **7**, mitochondrial ornithine transporter; **8**, arginase

Hyperornithinaemia due to ornithine aminotransferase (OAT) deficiency results in gyrate atrophy of the choroid and retina (GA). Although the progression of the retinal degeneration is highly variable, most GA patients lose all functional vision in middle age (45-65 years). Treatment includes an arginine-restricted diet and a trial of pharmacological doses (250-500 mg/day) of pyridoxine (vitamin B_6). Long-term compliance with an arginine-restricted diet, especially when started at a young age, can reduce ornithine accumulation and slow visual loss and chorioretinal degeneration. Creatine supplementation may be indicated to replenish tissue levels, but this question has not yet been adequately addressed. Rarely, OAT-deficient neonates present with hyperammonaemic encephalopathy due to impaired urea cycle function caused by substrate limitation, with associated hypoargininaemia and hypoornithinaemia. These infants respond to arginine supplementation. In the *hyperornithinaemia, hyperammonaemia, and homocitrullinuria (HHH) syndrome* there is a wide spectrum of clinical manifestations, most of which appear to be related to intermittent episodes of hyperammonaemia.

Progressive spastic paraparesis is often a late complication. HHH patients have hyperornithinaemia associated with episodic hyperammonaemia and increased urinary excretion of homocitrulline and orotic acid. HHH is an autosomal recessive trait resulting from a defect in the importation of ornithine into the mitochondria, with resulting impairment of urea cycle function. Treatment includes protein restriction and citrulline supplementation.

A newly recognised disorder, *P5C synthase (P5CS) deficiency*, has been described in two families with a neurocutaneous syndrome with lax skin, developmental delay, joint laxity and bilateral cataracts. The metabolic phenotype includes mild hyperammonaemia, hypoornithinaemia, hypocitrullinaemia, hypoargininaemia and hypoprolinaemia. This disorder underscores the importance of recognising low levels of amino acids as markers of metabolic disease. A second newly recognised disorder in these pathways, deficiency of P5C reductase (encoded by *PYCR*), has been shown to produce a cutis laxa-like phenotype but has not yet been well-characterised metabolically.

22.1 Hyperornithinaemia Due to Ornithine Aminotransferase Deficiency (Gyrate Atrophy of the Choroid and Retina)

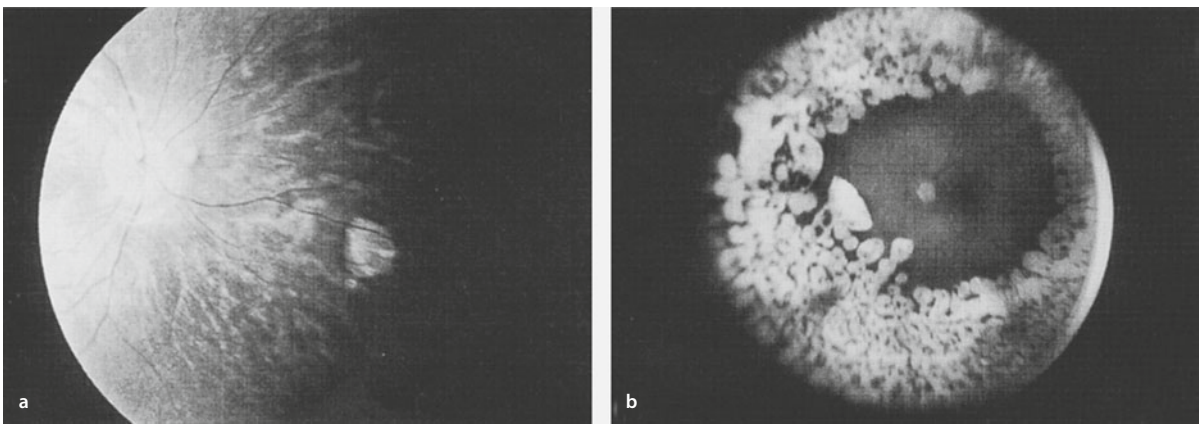
22.1.1 Clinical Presentation

The initial symptoms, which include myopia followed by night blindness, usually occur 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 (GA) is highly specific and is illustrated in ◘ Fig. 22.2.

The chorioretinal degeneration in GA 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 B_6 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 narrower than interfamilial variation [2]. Vitreous haemorrhage causing sudden loss of vision is a rare complication [3]. Most patients have normal intelligence consistent with other family members, although one report suggests an increased incidence of intellectual disability [1, 4].

A few patients have presented in the neonatal period with poor feeding, failure to thrive, symptomatic hyperammonaemia and orotic aciduria [5-8].

Post-mortem histopathological study of the retina in a pyridoxine-responsive patient showed focal areas of photoreceptor atrophy with adjacent retinal pigment epithelial hyperplasia [9]. Electron microscope studies revealed abnormal mitochondria in the corneal endothelium and the nonpigmented ciliary epithelium and similar, but less severe, abnormalities in the photoreceptors [9]. 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 in some patients, abnormal ultrastructure of hepatic mitochondria [10] and peculiar fine, sparse, straight hair with microscopic abnormalities [11]. Early degenerative and atrophic brain changes that were not age related were found by magnetic resonance



◘ **Fig. 22.2 a, b.** Fundoscopic appearances of the chorioretinal atrophy showing **a** early and **b** advanced changes

imaging (MRI) of the brain [12], and evidence of peripheral nervous system involvement [13] was noted in half the patients studied.

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22.1.2 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 (alternatively, ornithine ketoacid transaminase, OKT) [14]. The enzyme deficiency has been demonstrated in liver, muscle, hair roots, cultured skin fibroblasts and lymphoblasts [3]. 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 B_6).

Neonates who have presented with increased blood ammonia have low levels of plasma ornithine, citrulline and arginine in their first months, with hyperornithinaemia developing later in life [5-8]. 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 [8]. 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 [8]. 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 GA have reduced levels of creatine in blood, urine and muscle [12] as a result of ornithine inhibition of glycine transamidinase and the subsequent reduction of creatine biosynthesis (◘ Fig. 22.1). Brain NMR spectroscopy studies show reduced creatine content [13]. Subnormal levels of serum creatinine in GA reflect the reduction in total body creatine. In contrast to other series, which find normal intellect in adults with GA [1], a recent study in seven French paediatric GA patients revealed a high prevalence of neurological impairment [4]. The authors speculated that these phenotypic features could be related to secondary brain creatine deficiency [4]. This possibility should be carefully evaluated in future studies to consider possible complications of neonatal hyperammonaemia as an alternative explanation.

22.1.3 Genetics

GA is an autosomal recessive disorder and has been described in patients from various ethnic backgrounds, but its incidence is highest in the Finnish population [14]. Intermediate levels of OAT activity are observed in skin fibroblasts from obligate heterozygotes for both pyridoxine-nonresponsive and pyridoxine-responsive variants.

OAT is located at 10q26. More than 70 mutations have been defined in GA 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 [1].

22.1.4 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 in GA 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. Homocitrulline can be mistaken for methionine in some amino acid analysers (◘ Table 22.1). Neonatal GA can be difficult to distinguish from OTC deficiency, as plasma levels of ornithine, arginine, and citrulline are reduced in both disorders. Since hyperornithinaemia is not present in all (or perhaps any) neonates with GA, newborn screening using this as a marker will be unreliable. The levels of ornithine and other metabolites are not well described in neonates with HHH.

For confirmation of the diagnosis, direct assay of OAT activity can be performed in extracts of cultured skin fibroblasts or lymphoblasts. When the mutation is

Table 22.1. Differential diagnosis of disorders involving ornithine metabolism

	OAT deficiency	P5CS deficiency	HHH syndrome	OTC deficiency
Major clinical findings	Gyrate atrophy of the choroid and retina **Neonatal form:** Failure to thrive Encephalopathy	Mental retardation Lax and wrinkled skin Joint laxity Bilateral cataracts Peripheral neuropathy	Mental retardation Episodic lethargy and ataxia Seizures Coagulopathy	Neonatal onset of coma Mental retardation Episodic lethargy and ataxia Aversion to protein foods
Inheritance	Autosomal recessive	Autosomal recessive	Autosomal recessive	X-linked
Major biochemical changes				
Plasma ammonia	Increased in neonatal form	Increased during fasting or normal	Increased	Increased
Plasma ornithine	Increased Low in neonatal form	Low	Increased	Normal
Plasma citrulline and arginine	Low in neonatal form	Low	Normal	Low
Plasma proline	Normal	Low	Normal	Normal
Urine homocitrulline	*	Normal	Increased	Normal
Urine orotic acid	Normal Increased in neonatal form	Normal	Increased	Increased

HHH, hyperornithinaemia, hyperammonaemia, homocitrullinuria; *OAT*, ornithine aminotransferase; *OTC*, ornithine transcarbamoylase; *PC5S*, Δ^1-pyrroline-5-carboxylate synthase.* [1]Increased urine homocitrulline detected, but it was unclear whether it was an artefact from formula feeding

known, molecular analysis is appropriate for prenatal diagnosis and carrier detection.

22.1.5 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) [15-17]. 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 [17]. 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 GA. Infants with GA 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% [18-20]. 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 mmol/l and showed slower loss of visual function after 13.9 years than 10 patients not on the diet [21]. 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 [22]. 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 [23].

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 GA has shown that a trial of dietary arginine restriction completely prevented the appearance of retinopathy at the age when untreated mice developed GA [8]. This observation validates the efficacy of reduction in ornithine accumulation by arginine restriction in GA and emphasises the importance of early diagnosis and early treatment.

Other therapeutic approaches applied in small numbers of patients have included supplementation of proline [19], creatine [15, 24] and lysine [25]. 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 GA 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 women with GA [21]. 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.

22.2 Hyperornithinaemia, Hyperammonaemia and Homocitrullinuria (HHH) Syndrome

22.2.1 Clinical Presentation

The clinical manifestations in the HHH syndrome cover a broad spectrum, with some related to episodic hyperammonaemia (◘ Table 22.1) [25]. Ocular abnormalities are notably absent. 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 [26, 27]. Persistent or recurrent liver dysfunction was the presenting symptom in over a third of patients in a series of French-Canadian patients [28]. Also, severe but reversible hepatocellular necrosis and acute hepatitis-like episodes have recently been reported [28-30], suggesting that HHH should be added to the list of metabolic disorders causing liver failure. Coagulopathy, especially factor VII and X deficiencies, has been reported in several patients [31, 32], Variable intellectual impairment and/or progressive spastic paraparesis are often late complications, with no obvious relationship to age at diagnosis and/or compliance with treatment [27, 28]. Abnormal neuroimaging studies, including stroke-like lesions, have been described [33].

Mildly affected adult patients may have apparently normal intelligence. Two adult siblings originally attracted attention because of episodic neurological and psychiatric disturbances with protein intolerance [34].

22.2.2 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. 22.1), resulting in a functional deficiency of both OTC and OAT activities (◘ Table 22.1). 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. 22.1) and formation of orotic acid secondary to excess flux down the pyrimidine biosynthetic pathway (► Chapter 36, ◘ Fig. 36.3).

22.2.3 Genetics

The HHH syndrome is more common in French-Canadians than in other ethnic groups. Inheritance is autosomal recessive. The gene (*ORNT1* or *SLC25A15*) encoding the transporter protein is located at 13q14. The common mutant allele in HHH patients of French-Canadian origin is F188Δ, a 3-bp inframe deletion [35]. Even in homozygotes for this deletion there is considerable phenotypic variability, and also for other patients there is no clear-

cut genotype-phenotype correlation [28, 36]. The R197X mutation has been reported in multiple Japanese patients [37]. Obligate heterozygotes are clinically normal and cannot be identified by biochemical studies.

22.2.4 Diagnostic Tests

The HHH syndrome can be differentiated from other hyperammonaemic syndromes by laboratory findings (◘ Table 22.1). The triad of hyperornithinaemia, hyperammonaemia and homocitrullinuria is pathognomonic. The plasma ornithine concentration is elevated to 3-10 times normal and tends to be somewhat lower than that seen in GA patients. Despite a functional deficiency of OTC activity, plasma citrulline is normal in the HHH syndrome.

In addition to homocitrullinuria, urine amino acid screening shows increased ornithine and hyperdibasic amino aciduria when the plasma ornithine concentration is above 400 mol/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 [38]. 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 [34], as in patients with primary OTC deficiency (▶ Chapter 20).

The metabolic defect can be detected with an assay measuring ^{14}C-L-ornithine incorporation into protein using fibroblast monolayers [39]. Fibroblasts from both GA and HHH patients fail to incorporate ^{14}C derived from ornithine into protein, but OAT activity (normal in HHH, deficient in GA) distinguishes the two disorders. The method of choice for prenatal diagnosis in couples of known genotype is mutation analysis.

22.2.5 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 20). In general, a low-protein diet, sodium benzoate and/or citrulline supplementation have been effective in achieving biochemical control for most patients. Arginine should only be supplemented with caution, since it may lead to hyperargininaemia, which may contribute to the development of spastic paraparesis. One patient with neonatal onset of moderate hyperammonaemia responded well to treatment and had normal growth and development at 18 months of age [40]. Treatment has not prevented the late development of spastic gait [27], although the authors' personal experience includes multiple patients who have been treated with citrulline supplementation and mild dietary protein restriction for more 20 years with no progression of neurological abnormalities.

Hyperammonaemia during pregnancy and post partum is a potential risk in women with the HHH syndrome. One patient developed hyperammonaemia 1 day post partum after each of two pregnancies (V. Shih, unpublished observation). This is probably due to endogenous nitrogen load from uterine involution. It is thus advisable to exercise caution in the postpartum dietary management of HHH patients. Another woman was treated during pregnancy with lactulose and arginine to reduce blood ammonia [41]. Offspring of both women and men with HHH syndrome have been apparently normal.

22.3 Δ¹-Pyrroline-5-Carboxylate Synthase Deficiency

22.3.1 Clinical Presentation

The clinical information on this disorder is limited to that recorded in two consanguineous families with respectively two and four affected siblings [42, 43]. The first two patients aroused clinical attention in early infancy because of developmental delay, lax skin and joints, muscular hypotonia and failure to thrive [42]. 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 (IQ 50). 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.

The four affected children of the second family showed pronounced abnormalities of connective tissue, most notably wrinkly skin over the hands, feet, abdomen, chest and face, which disappeared with age and was no longer clinically evident by adolescence [43]. Wounds healed normally, and no excessive scarring was noted. Joint dislocations, mild proportionately short stature and global developmental delay were also noted. Bilateral cataracts were present in one patient.

22.3.2 Metabolic Derangement

The metabolic phenotype described in the first but not in the second family includes mild hyperammonaemia, hypoornithinaemia, hypocitrullinaemia, hypoargininaemia and hypoprolinaemia, a pattern of metabolic abnormalities consistent with impaired proline and ornithine synthesis due to deficiency of Δ^1-pyrroline-5-carboxylate synthase (P5CS). This enzyme catalyses an essential step in the pathways by which proline, ornithine and arginine are synthesised from glutamate. 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 [44].

The paradoxical fasting hyperammonaemia reported in one of the 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.

22.3.3 Genetics

Deficiency of P5CS is inherited as an autosomal recessive trait. Only two affected families have been reported, one Algerian and one New Zealand Maori, both with consanguineous parents and each homozygous for a missense mutation in *ALDH18A1* encoding P5CS, p.R84Q and p.H784Y, respectively. R84Q alters a conserved residue in the P5CS γ-glutamyl kinase domain and dramatically reduces P5CS activity when expressed in mammalian cells [44]. H784 lies within a previously unappreciated, conserved C-terminal motif in P5CS. In an in vivo assay of flux through this metabolic pathway, proline and ornithine biosynthetic activity of P5CS was not affected by the H784Y substitution, suggesting that P5CS may possess additional uncharacterised functions that affect connective tissue and/or central nervous system [43].

22.3.4 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.

P5CS activity is undetectable in control fibroblasts [45]. 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 [42], corroborating the biological significance of the metabolic block at the level of P5CS in vivo.

22.3.5 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 an amino acid cocktail, such as one containing citrulline, arginine, ornithine and proline.

22.4 Δ^1-Pyrroline-5-Carboxylate Reductase Deficiency

Recently, mutations in the gene encoding P5C reductase (*PYCR1*) have been described in patients with progeroid appearance, lax and wrinkled skin apparent at birth, joint laxity and mental retardation, supporting the assumption of a significant role for proline biosynthesis in connective tissue and in normal intellectual development [46, 47]. Cells and tissues from these individuals display increased apoptosis in response to oxidative stress [46]. Serum proline levels in these patients were normal. This disorder is inherited as an autosomal recessive trait.

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Cerebral Organic Acid Disorders and Other Disorders of Lysine Catabolism

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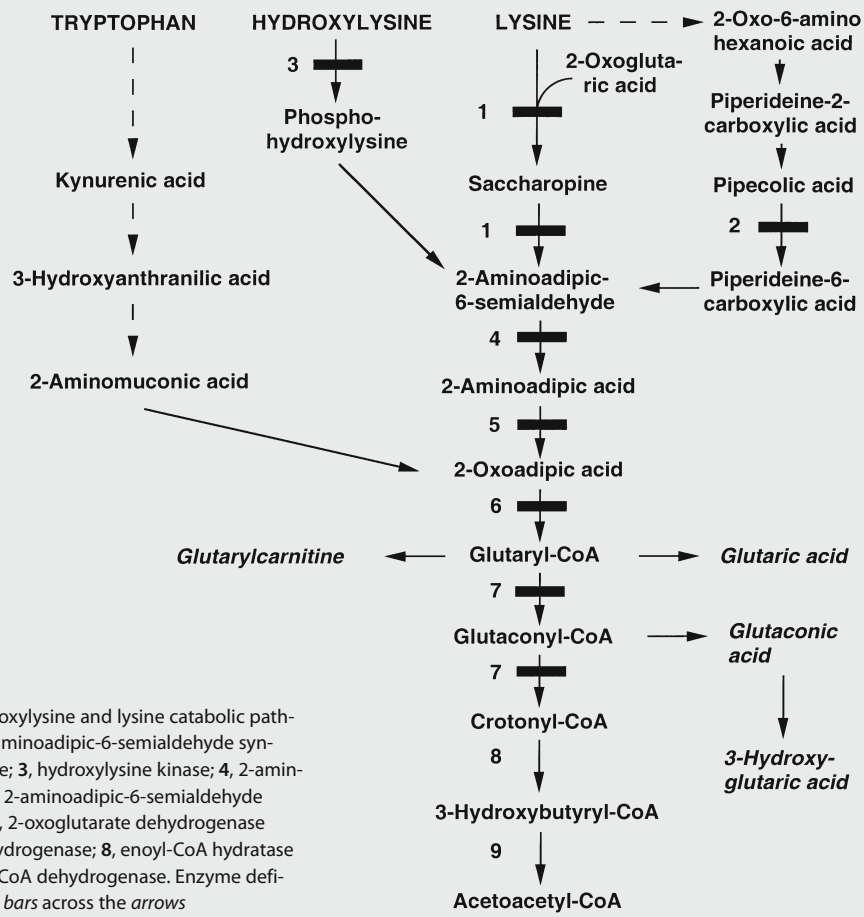
Catabolism of Lysine, Hydroxylysine, and Tryptophan

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. 23.1). 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 enzyme, pipecolic acid oxidase (enzyme 2); this pathway, however, is regarded as the major route of lysine catabolism in the brain. 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 converted to glutaryl-CoA by the 2-oxoglutarate dehydrogenase complex (enzyme 6), which is then 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 (◘ Fig. 13.1) via electron transfer protein (ETF)/ETF-dehydrogenase (ETF-DH). Crotonyl-CoA subsequently enters the distal pathway of fatty acid oxidation, being converted to 3-hydroxybutyryl-CoA by enoyl-CoA hydratase (crotonase, enzyme 8) and



◘ **Fig. 23.1.** Tryptophan, hydroxylysine and lysine catabolic pathways. *CoA*, coenzyme A. **1**, 2-aminoadipic-6-semialdehyde synthase; **2**, pipecolic acid oxidase; **3**, hydroxylysine kinase; **4**, 2-aminoadipate aminotransferase; **5**, 2-aminoadipic-6-semialdehyde dehydrogenase (antiquitin); **6**, 2-oxoglutarate dehydrogenase complex; **7**, glutaryl-CoA dehydrogenase; **8**, enoyl-CoA hydratase (crotonase); **9**, 3-hydroxyacyl-CoA dehydrogenase. Enzyme deficiencies are indicated by *solid bars* across the *arrows*

then to acetoacetyl-CoA by 3-hydroxyacyl-CoA dehydrogenase (enzyme 9, ► Chapter 13). From the five distinct enzyme deficiencies identified in the degradation of lysine, only enzymes 4, 6 and 7 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 13. Pipecolic acid oxidase (enzyme 2) is discussed with peroxisomal disorders in ► Chapter 41, 2-aminoadipic-6-semialdehyde dehydrogenase (antiquitin, enzyme 4) deficiency in ► Chapter 29, and 2-oxoglutarate dehydrogenase deficiency (enzyme 6) in ► Chapter 12.

L-2- and D-2-Hydroxyglutaric aciduria type I have recently been shown to be caused by deficiencies of specific FAD-dependent dehydrogenases, whereas D-2-hydroxyglutaric aciduria type II is caused by deficient mitochondrial isocitrate dehydrogenase 2. 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.

Ten inborn errors of metabolism are described in this chapter. Four of them, *hyperlysinaemia/saccharopinuria, hydroxylysinuria, 2-amino-/2-oxoadipic aciduria,* and *glutaric aciduria type III*, may be devoid of clinical significance, but some patients are retarded and show variable neurological abnormalities.

Glutaric aciduria type I causes severe neurometabolic disease. The first months may be uneventful with only subtle neurological abnormalities and/or macrocephaly, but neuroimaging may reveal an immature pattern of gyration and myelination in combination with periventricular pseudocysts and temporal hypoplasia, which may regress, or subdural haemorrhages. By age 3 years most untreated patients suffer an acute encephalopathy resulting in irreversible destruction of susceptible brain regions, in particular the striatum, a complex movement disorder with predominating dystonia superimposed on axial hypotonia and, ultimately, often reduced life expectancy. A low-lysine diet, administration of L-carnitine and timely vigorous emergency treatment during intercurrent illness is able to prevent or at least halt the neurological disease in the majority of neonatally identified patients.

L-2-Hydroxyglutaric aciduria shows an insidious onset with delay of unsupported walking and speech, febrile convulsions and macro- or microcephaly. Over the years, severe mental retardation and cerebellar ataxia develop, with or without dystonia, pyramidal signs and seizures. Neuroimaging reveals dilated ventricles, signal abnormalities of subcortical and periventricular white matter, and characteristic signal changes in the globus pallidus and dentate nuclei. A few patients have been reported as having brain tumours, such medulloblastoma, glioblastoma and primitive neuroectodermal tumours.

D-2-Hydroxyglutaric aciduria can cause severe early-onset epileptic encephalopathy with neonatal seizures, lack of psychomotor development, cardiomyopathy and early death. Some patients exhibit milder neurological symptoms, such as mild developmental delay, delayed speech and febrile convulsions. Recent studies have elucidated genetic heterogeneity distinguishing type I and II.

N-Acetylaspartic aciduria (synonyms: aspartoacylase deficiency, spongy degeneration of the brain, Van Bogaert-Bertrand disease, Canavan disease) is an infantile degenerative disease primarily affecting the cerebral white matter. It commonly manifests with poor head control and hypotonia at 2-4 months, megalocephaly, marked developmental delay, optic nerve atrophy, progressive spasticity, opisthotonic posturing, seizures and death in childhood.

Hypoacetylaspartia, caused by aspartate *N*-acetyltransferase deficiency and characterised by the absence of brain NAA, has been described in a single patient with truncal ataxia, marked developmental delay, seizures, and microcephaly.

23.1 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, 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. 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 19), 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. 23.2, Fig. 23.3). 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.

23.2 Hyperlysinaemia/ Saccharopinuria

23.2.1 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.

23.2.2 Metabolic Derangement

Hyperlysinaemia/saccharopinuria is caused by deficiency of the bifunctional protein 2-aminoadipic semialdehyde synthase. 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 results 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.

Hyperlysinuria can also result from impaired renal tubular transport, often as part of a genetic transport defect of dibasic amino acids (► Chapter 26), and in this situation it occurs without hyperlysinaemia.

23.2.3 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].

23.2.4 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-aminoadipic 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].

23.2.5 Treatment and Prognosis

Long-term dietary restriction of lysine has no benefit. As patients 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.

23.3 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.

23.4 2-Amino-/2-Oxoadipic Aciduria

23.4.1 Clinical Presentation

2-Amino-/2-oxoadipic aciduria is probably of no clinical significance. Over 20 patients are known, more than half of whom are asymptomatic [7] (G.F. Hoffmann, unpublished observation). Symptoms include psychomotor retardation, muscular hypotonia, epilepsy, ataxia and failure to thrive, but is likely that these are coincidental findings.

23.4.2 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. Normally 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, most probably by the oxoglutarate dehydrogenase complex – analogous to the oxidative decarboxylation of 2-oxoglutarate to succinyl-CoA in the tricarboxylic acid cycle.

The metabolic origin of 2-amino/2-oxoadipic aciduria remains obscure.

23.4.3 Genetics

Autosomal recessive inheritance is implied by the pedigrees and by the finding that parents can not be biochemically differentiated from controls. The molecular basis of this disease, however, remains to be elucidated.

23.4.4 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].

23.4.5 Treatment and Prognosis

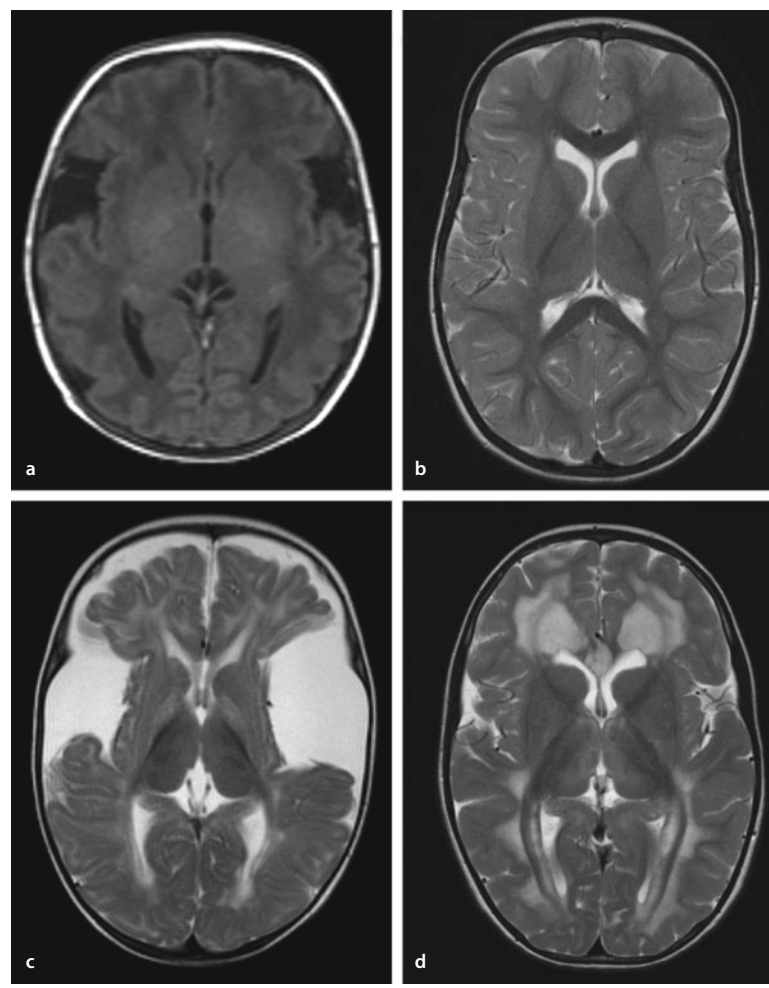
As 2-amino-/2-oxoadipic aciduria is likely to be a non-disease; it does not determine morbidity or mortality. Patients do not suffer from metabolic decompensations, and specific interventions during intercurrent illnesses do not appear necessary. Administration of pharmacological doses of vitamins B_1 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.

23.5 Glutaric Aciduria Type I

23.5.1 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 to-

mography (CT; ◘ Fig. 23.2a-d) and/or a complex extrapyramidal syndrome of predominantly dystonia, orofacial dyskinesia and dysarthria superimposed on axial hypotonia. Choreic movements may also be observed [8-11]. 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. 23.2a), 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. 23.2b). 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].



◘ **Fig. 23.2 a-d.** MRI findings in patients with glutaric aciduria type I. **a** T_1-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** T_2-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** T_2-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** T_2-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)

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 [10]. MRI reveals striatal injury spreading in a dorsoventral direction (◘ Fig. 23.2c). 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 or only mild ones. A severe dys-/hypotonic movement disorder then 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. A generalised cerebral atrophy emerges, giving rise to pyramidal tract signs and mental retardation. 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 neuropathology 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 recently been described in five previously unaffected adolescent/adult patients presenting with signal changes in the white matter but unaffected basal ganglia [12], suggesting an additional disease variant (◘ Fig. 23.2d).

23.5.2 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 (◘ Fig. 23.1). 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, which is indicative of disturbed mitochondrial function.

The mechanism of age-specific destruction of specific cerebral structures in glutaric aciduria type I has 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 [16-19]. 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 is suggested that disturbed cerebral haemodynamics, such as disturbed autoregulation and regional perfusion pressure gradients, adds to the metabolic toxicity of this disease [14].

23.5.3 Genetics

Glutaric aciduria type I is an autosomal recessive disorder caused by pathogenic mutations in the *GCDH* gene located on 19p13.2. Results of newborn screening programmes in various regions and cohorts worldwide give an overall mean frequency of 1:100,000 [15]. The disease is very frequent in certain communities, such as the Amish people in Pennsylvania (homozygous for p.A421V, incidence of 1 in 300-400 newborns) , the Saulteaux/Ojibway Indians 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.E365K).

More than 200 different disease-causing mutations in the *GCDH* gene have been identified so far [20]. There is a correlation between genotype and biochemical pheno-

type in that specific mutations with significant residual enzyme activity may be associated with low excretions of metabolites in compound heterozygous patients who carry a severe mutation on the other allele. However, no correlation between genotype and clinical phenotype has yet been found [10]. Single common mutations are found in genetically homogenous communities (see above), but glutaric aciduria type I in general is genetically quite heterogeneous: the most frequent mutation in caucasians, p.R402W, has been identified on 10–20% of alleles [20].

23.5.4 Diagnostic Tests

Patients with glutaric aciduria type I have generally been diagnosed by urinary organic acid analysis [21]. 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 [22]. Application to analyses of blood spots (Guthrie cards) has enabled the recent inclusion of glutaric aciduria type I into the neonatal screening programmes of some 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. 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 [21]. 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 13) 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 [23].

Carrier detection is possible by enzyme assay, though the results are not always unequivocal [23], and by molecular means in families in which the mutations are already known [20]. Reliable *prenatal diagnosis* can be offered by enzyme assay [24], determination of glutaric acid by stable isotope dilution gas chromatography–mass spectrometry (GC-MS) assay in amniotic fluid [21], and by molecular analysis [20]. Evidence-based recommendations for the diagnosis of glutaric aciduria type 1 have been published recently [24].

23.5.5 Treatment and Prognosis

Three 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, if diagnosis is made early and treatment is started before manifestation of acute encephalopathic crises. Early diagnosis and treatment of the asymptomatic child is essential, as current therapy has little effect once brain injury has occurred. Following an encephalopathic crisis the majority of patients remained handicapped and have a reduced life expectancy. However, even with early diagnosis 10–35% of patients do not benefit, or benefit only incompletely, from the current management [9, 15, 25].

The following therapeutic measures are generally employed and are recommended by a recent guideline [24].

1. Emergency Treatment. Emergency treatment must start before the onset of severe neurological signs. During intercurrent illnesses, especially gastrointestinal infections, 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 [24]. 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, 26, 27].

2. Oral Supplementations with Carnitine and Riboflavin. Carnitine should be supplemented lifelong to prevent secondary carnitine depletion. Furthermore, lack of car-

Table 23.1. Maintenance therapy in patients with glutaric aciduria type I. (After [27])

		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; intake of 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

nitine supplementation has been associated with high mortality [10]. Riboflavin responsiveness appears to be extremely rare, and the therapeutic benefit of riboflavin is unproven. Furthermore, there is no standardised protocol to test for riboflavin responsiveness.

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 23.1). Application of lysine-free 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, 24]. 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 [24]. 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 or are 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, seating and speech specialists, and providers of communication aids should be consulted to provide multi-professional support for children with movement disorders.

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 improved the short-term outcome of affected individuals by decreasing the frequency of children with striatal injury who would have developed a complex movement disorder [15, 26, 25].

23.6 Glutaric Aciduria Type III

23.6.1 Clinical Presentation

Glutaric aciduria type III is an autosomal recessive metabolic abnormality with unknown incidence. It is a clinically benign condition [28].

23.6.2 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). The original hypothesis was that glutaric aciduria type III is caused by peroxisomal glutaryl-CoA oxidase deficiency. However, this enzyme has not been identified in humans.

23.6.3 Genetics

Recently, pathogenic mutations in the *C7orf10* gene located on 7p14 were identified as causative for glutaric aciduria type III. The *C7orf10* gene product has a putative mitochondrial targeting sequence and a CoA transferase domain. It may function as part of a multiunit enzyme complex in the mitochondrial lysine and tryptophan-degradative pathway [29].

23.6.4 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.

23.6.5 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.

23.7 L-2-Hydroxyglutaric Aciduria

23.7.1 Clinical Presentation

Most patients with L-2-hydroxyglutaric aciduria follow a characteristic disease course [30-32]. 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 [32].

In L-2-hydroxyglutaric aciduria the neuroimaging findings are very specific [30, 31]. 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. 23.3a, b) on T_2-weighted images. In some patients different types of malignant brain tumours, such as medulloblastoma, glioblastoma multiforme, astrocytoma, and primitive neuroectodermal tumour, have been reported [33].

23.7.2 Metabolic Derangement

Quantitative analysis of organic acids has revealed elevations of L-2-hydroxyglutarate in CSF, plasma and urine [34]. In addition, a number of hydroxydicarboxylic acids (glycolate, glycerate, 2,4-dihydroxybutyrate, citrate and isocitrate) were only found elevated in CSF. Another consistent biochemical finding is an increase of lysine in blood and CSF.

Elevation of L-2-hydroxyglutarate is caused by an inherited deficiency of FAD-linked 2-hydroxyglutarate dehydrogenase, a mitochondrial enzyme converting L-2-hydroxyglutarate to 2-oxoglutarate [35]. L-2-Hydroxyglutarate has no known functions, but its formation may be simply the result of the fact that L-malate dehydrogenase is not absolutely specific for oxaloacetate. L-Malate dehydrogenase may slowly catalyse the reduction of 2-oxoglutarate, the structural homologue of oxaloacetate, to L-2-hydroxyglutarate. The latter is normally reoxidised to 2-oxoglutarate by L-2-hydroxyglutarate dehydrogenase [36]. 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 faulty metabolite, or 'metabolite repair'.

23.7.3 Genetics

L-2-Hydroxyglutaric aciduria is an autosomal recessive disorder. Mutations in the *L2HGDH* (*C14orf160/duranin*)

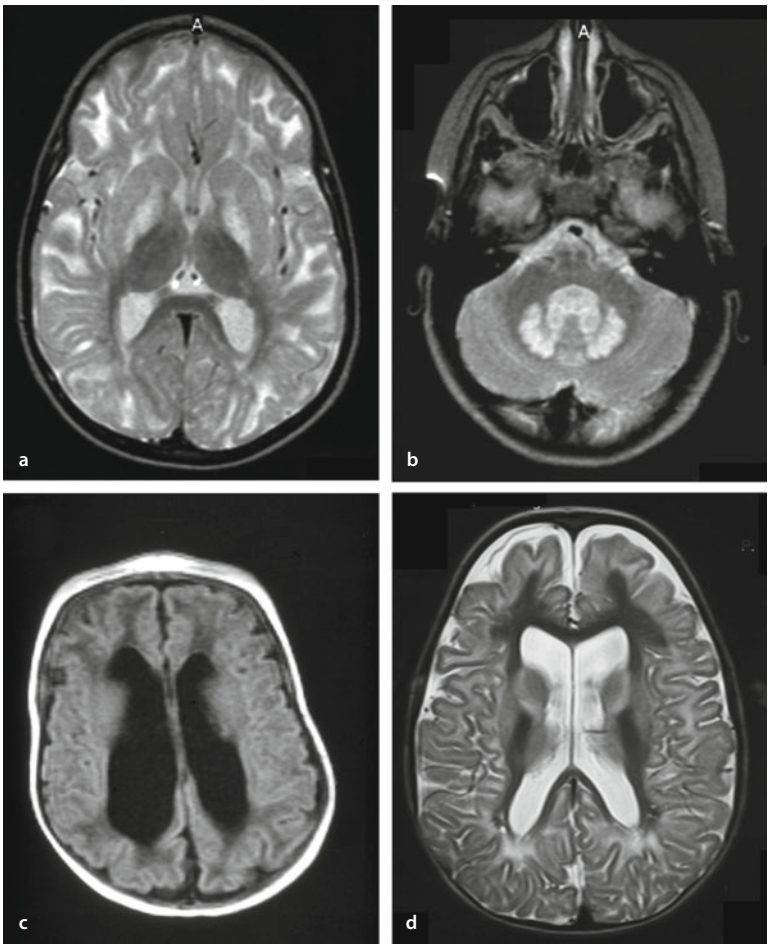


Fig. 23.3 a-d. MRI findings in patients with other cerebral organic acidurias. **a**, **b** Axial T_2-weighted MRI of a 8.5-year-old boy with L-2-hydroxyglutaric aciduria, illustrating characteristic involvement of subcortical white matter (also affecting the U fibres) and globus pallidus (**a**), and 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

gene located on 14q22.1 have been identified as causative for this disease [37].

23.7.4 Diagnostic Tests

L-2-Hydroxyglutarate is found elevated in all body fluids [30, 38]. 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 [31, 38] and by molecular diagnosis.

23.7.5 Treatment and Prognosis

The use of riboflavin treatment has been reported in a few patients to have led to a partial improvement of neurological symptoms and reduced urinary excretion of L-2-hydroxyglutarate [39]. Epilepsy can generally be controlled by standard medications. The oldest known patients are over 30 years of age. They are bedridden and severely retarded.

23.8 D-2-Hydroxyglutaric Aciduria

23.8.1 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 and no symptoms at all [40]. An international survey revealed a continuous spectrum between these extremes, with most patients suffering from a severe earl- onset epileptic encephalopathy, while a substantial subgroup showed mild symptoms or were even asymptomatic [41]. Clinical and neuroradiological symptoms of the severely affected patients were quite

uniform. Severe, often intractable seizures started in early infancy. The babies were severely hypotonic. Conscious levels varied from irritability to stupor. Cortical blindness was uniformly present, and psychomotor development appeared almost absent. A third of the severely affected patients suffered from cardiomyopathy. 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. 23.3c).

23.8.2 Metabolic Derangement

Patients show moderately to highly elevated levels of D-2-hydroxyglutarate in all body fluids with no apparent correlation to the clinical phenotype. In addition Krebs cycle intermediates are found to be elevated in the urine of some patients, as well as GABA in CSF [41]. In half of the patients, D-2-hydroxyglutaric aciduria has been related to deficient D-2-hydroxyglutarate dehydrogenase, an enzyme that converts D-2-hydroxyglutarate to 2-oxoglutarate [42, 43]. The enzyme is homologous to FAD-dependent D-lactate dehydrogenase. Recently, mutated mitochondrial isocitrate dehydrogenase 2 has been identified in the other half of patients with D-2-hydroxyglutaric aciduria [44]. This mutation disables the enzyme's normal function to convert d-isocitrate into 2-oxoglutarate but confers on it 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) [45, 46]. 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.

23.8.3 Genetics

Autosomal recessively inherited mutations in the human *D2HGDH* gene located on 2p25.3 have been identified as the molecular cause of D-2-hydroxyglutaric aciduria [43]. 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 [47]. Significantly lower D-2-hydroxyglutarate concentrations in body fluids were observed in mutation-positive than in mutation-negative patients. Recently, autosomal dominant germline mutations of the *IDH2* gene located on 15q26.1 [44] were identified as a second molecular cause of D-2-hydroxyglutaric aciduria. A high frequency of de novo *IDH2* mutations was found. Based on this, a novel classification has been suggested, i.e. D-2-hydroxyglutaric aciduria type I for autosomal recessive D-2-hydroxyglutaryl-CoA dehydrogenase deficiency, and D-2-hydroxyglutaric aciduria type II for patients with autosomal dominant IDH2 deficiency.

23.8.4 Diagnostic Tests

L-2- and D-2-Hydroxyglutaric acid cannot be differentiated by conventional GC-MS analysis. The chromatographic separation of these enantioners 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 [38, 41, 47]. 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 [38].

A few patients with combined D- and L-2-hydroxyglutaric aciduria have been described [48]. It is unclear whether they represent yet another clinical and/or biochemical entity. However, in these patients *prenatal* diagnosis is not reliable using metabolite determination by stable isotope dilution GC-MS assay in amniotic fluid [38].

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 distinguished by the urine organic acid profile (► Chapter 13).

23.8.5 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 de-

velopmental delay. In general the clinical course does not appear progressive, if affected children do not develop an early onset epileptic encephalopathy.

23.9 N-Acetylaspartic Aciduria (Canavan Disease)

23.9.1 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 [49]. 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. Death usually occurs within a few years, although survival in a vegetative or near-vegetative state may extend to the 2nd decade.

The most consistent findings on MRI studies are diffuse abnormalities of white matter [50]. 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. 23.3d).

The neuropathology of Canavan disease is characterised by a progressive loss of myelinated arcuate fibres [50]. 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.

23.9.2 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 [51]. 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 [52].

23.9.3 Genetics

N-Acetylaspartic aciduria is an autosomal recessive disease caused by pathogenic mutations in the *ASPA* gene located on 17pter-p13. It is a pan-ethnic disease with a higher frequency among Askenazi Jews, most of whom carry two specific mutations, a missense mutation, p.E285A, accounting for 84% of mutant alleles, and a nonsense mutation, p.Y231X, accounting for 13% [53]; the frequency of these two mutations makes carrier screening possible [54]. In non-Jewish patients the mutations are diverse and mostly private.

23.9.4 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 cases of white matter disease and can cause diagnostic confusion. Prenatal diagnosis can be problematic, as the assay of aspartoacylase in amniocytes is not reliable [55]. A combination of mutation analysis together with the exact quantitation of NAA in the amniotic fluid is recommended.

23.9.5 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 benefical [56]. 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 is 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 [57]. Adenoviral transfer of the *ASPA* gene to the brains of patients has been initiated [58]. 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 which expressed delivered genes were neurons, so that the entire oligodendroglial defect remains uncorrected, as has been demonstrated in the tremor rat model of Canavan disease [59]. Therefore, the prognosis for most affected individuals is still very poor, with death usually occurring in the first decade of life.

23.10 Hypoacetylaspartia

A single patient has been described with marked developmental delay and secondary microcephaly with truncal ataxia, seizures, and behaviour abnormalities on follow-up, in whom ^{1}H-MRS had revealed the absence of NAA signal [60]. A defect of L-aspartate *N*-acetyltransferase, the enzyme that synthesises NAA, was suspected, but could not be confirmed because its molecular identity had remained unknown. Recently, a neuron-specific protein, NAT8L, was found to be responsible for NAA synthesis, and was mutated in the patient [61].

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Nonketotic Hyperglycinaemia (Glycine Encephalopathy)

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Glycine Metabolism

Glycine, the simplest of the amino acids, is abundant in nearly all animal proteins and enters into more biosynthetic routes than any other. Formation of glycine conjugates plays an important role in the detoxification of various compounds, including those that accumulate in certain inborn errors of metabolism. The catabolism of glycine involves several pathways, among which the glycine cleavage system (GCS) is of major importance. This multienzyme complex degrades glycine into NH_3 and CO_2, thereby also converting tetrahydrofolate to 5,10-methylene tetrahydrofolate. The latter compounds are also involved in the interconversion of serine and glycine, catalysed by serine hydroxymethyl transferase.

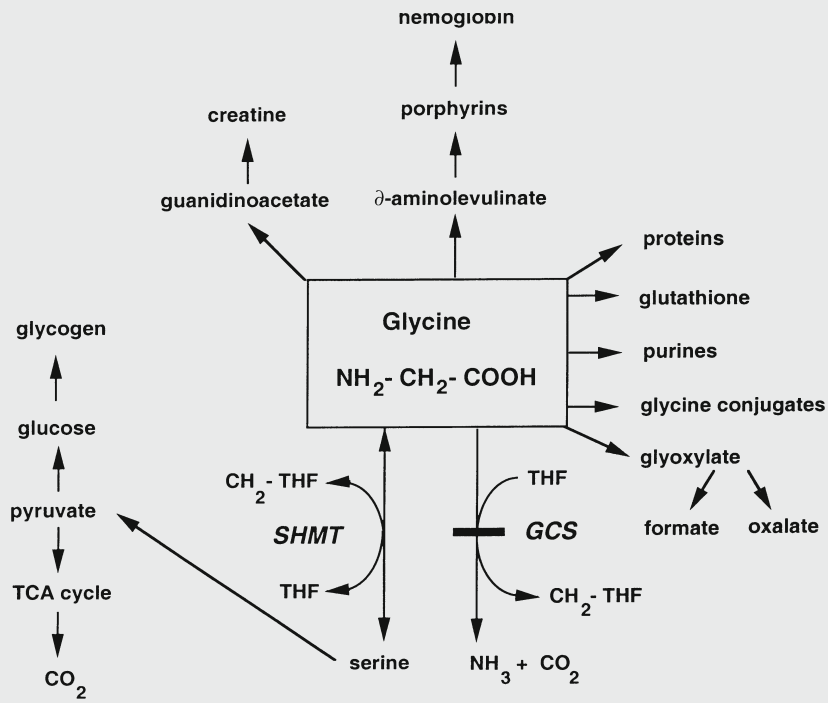

Fig. 24.1. Pathways of glycine metabolism. *CH2-THF*, 5,10-methylene tetrahydrofolate; *GCS*, glycine cleavage system; *SHMT*, serine hydroxymethyl transferase; *TCA*, tricarboxylic acid; *THF*, tetrahydrofolate. The enzyme defect is depicted by the *solid bar*

Nonketotic hyperglycinaemia (NKH) or glycine encephalopathy is an autosomal recessive disorder characterised by a rapidly progressive course in the neonatal period or early infancy. Symptoms include muscular hypotonia, seizures, apnoeic attacks, lethargy and coma. Most patients die within a few weeks, whilst survivors show severe psychomotor retardation. Increased glycine concentrations in plasma, urine, and cerebrospinal fluid are biochemical features of the disorder. The primary lesion is a defect in the glycine cleavage system (GCS) (Fig. 24.1). Although this was first demonstrated in the liver, involvement within the brain is responsible for the clinical expression. No specific treatment is available. Prenatal diagnosis is possible by determining the activity of GCS in chorionic villi and by molecular analysis.

24.1 Clinical Presentation

Nonketotic hyperglycinaemia (NKH) is usually classified into two main clinical types: neonatal and late-onset. The *neonatal type* is the most common. In the series of Tada and Kure [1], 28 of 32 cases (87%) presented in the newborn period.

24.1.1 Neonatal NKH

The phenotype is characteristic, so that the diagnosis can be suspected on the basis of the clinical and electroencephalographic (EEG) features. A severe encephalopathy, without ketosis or acidosis, occurs after an apparently

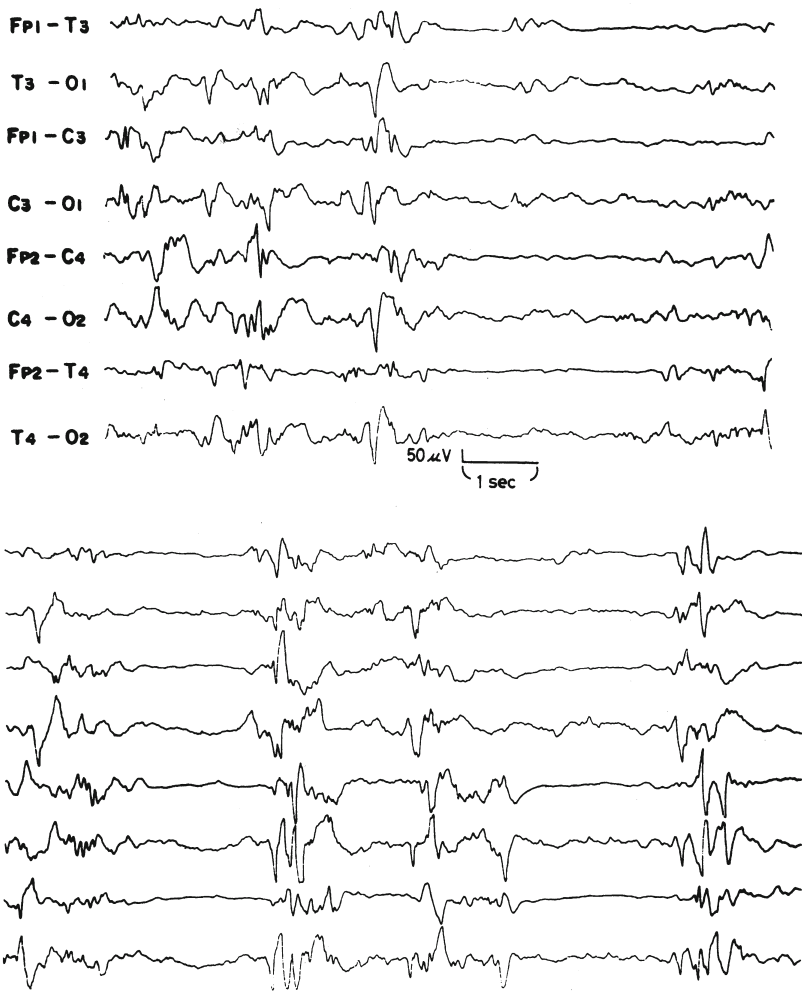

Fig. 24.2. Electroencephalogram of a 6-week-old patient with nonketotic hyperglycinaemia, showing a burst-suppression pattern

symptom-free interval, although some infants have brain malformations such as dysgenesis of the corpus callosum and gyral malformations [2], which may be detected prenatally [3] or evident at birth. Most, however, appear normal at birth but within the first few hours develop a progressive encephalopathy characterised by lethargy, axial and limb hypotonia and a depressed Moro response. Respiration becomes increasingly irregular, culminating in apnoeic attacks, by which time the infant is in a deep coma and exhibits myoclonus, and eventually tonic or clonic seizures. Hiccups are often present.

In the early hours of myoclonic activity the EEG may be normal [4], although in most instances before the first ictal events it shows a burst suppression (BS) pattern consisting of periods of high-amplitude activity lasting 1-3 Hz that arise periodically from a hypoactive background (Fig. 24.2) and without spatio-temporal differentiation. The bursts are mostly asynchronous over both hemispheres and comprise irregular sharp waves and spikes. This pattern disappears by the end of the 1st month of life, changing into hypsarrhythmia. Magnetic resonance imaging (MRI) shows callosal thinning, progressive cortical atrophy and delayed myelination, particularly in the parietal lobes, with high signal in the pyramidal tracts, middle cerebellar pedicles and dentate nuclei. Serial diffusion-weighted (DWI) and diffusion tensor imaging (DTI) show increased T_2-signal intensity and restricted diffusion consistent with vacuolating myelinopathy [5]. By about 17 months, diffusion restriction has disappeared, probably because of coalescence of myelin vacuoles. A decrease of fractional anisotropy is observed in the previously myelinated areas indicative of axonal loss. The development of acute hydrocephalus requiring shunting in early infancy has been reported [6]. On magnetic resonance spectroscopy, lactate and creatine are increased, and levels of *N*-acetylaspartate and myoinositol-glycine may be prognostic indicators [7]. An elevated glycine peak is evidenced on long-echo-time proton magnetic

resonance spectroscopy [8]. Pulmonary hypertension has been documented in four patients [9].

Neonatal hypotonia gradually evolves into spasticity. Most patients die at between 6 days and 5 years of age. Neuropathology shows spongiosis and myelin vacuolation. The patients who survive are severely retarded [10].

Transient neonatal NKH has been described in a few newborns with symptoms indistinguishable from those of the neonatal type [11, 12]. Plasma and CSF glycine concentrations are initially elevated to those seen in neonatal NKH, but return to normal by the end of the 2nd month. Intracranial bleeding in neonatal ischaemic encephalopathy may be a confounding factor, for both biochemistry and magnetic spectroscopy [13]. Most patients have had no neurological sequelae after 6 months to 13 years of follow-up. However, one had severe developmental delay at 9 months of age. A favourable outcome in this group of patients may be related to a high residual activity of the glycine cleavage system (GCS) [14] or heterozygosity [15] and vigorous therapeutic intervention with sodium benzoate and ketamine or dextrometorphan in the neonatal period, when the brain is particularly vulnerable to glycine. Glycine cleavage enzyme activity on liver biopsy may prove normal when the course is favourable [16]. One variant with the homozygous A802V mutation was characterised by the persistence of metabolic abnormalities, although the clinical condition improved dramatically [14]. The 2607C<A mutation was identified in a nine-patient kindred with mild clinical expression consisting in hypotonia, abnormal movements, convulsions and moderate mental retardation, with relative sparing of gross motor function, activities of daily living skills and receptive language. Aggression and irritability were prominent [17]. However, one responder remained dependent on ketamine and only experienced partial improvement while blood levels of glycine remained high [18]. The identification of this rare group opens major ethical issues about the indication for treatment in neonatal nonketotic hyperglycinaemia [19].

24.1.2 Late-onset NKH

In patients with the *late-onset* type, there are no abnormal symptoms or signs in the neonatal period, but thereafter nonspecific neurological symptoms varying in degree develop. The age of onset ranges from infancy to late adulthood. In adults NKH can present with paroxysmal choreic movement disorders, confusion triggered by fever or mental retardation with aggressive behaviour. Three Japanese cases, all retaining 6-8% activity of the GCS, exhibited infantile hypotonia, mental retardation and episodes of disturbed behaviour in childhood and adolescence (aggressiveness and intractable attention deficit hyperactivity disorder [ADHD]) [20]. Several patients suffered from pulmonary hypertension from the 1st months of life, with rapidly progressive neurological deterioration following vaccination, including hypotonia, pyramidal signs and loss of cognitive functions, but neither seizures nor EEG abnormalities [21]. One late-onset NKH patient with mild language delay and mental retardation was found to have nonketotic hyperglycinaemia following her presentation with acute encephalopathy and chorea shortly after the initiation of valproate therapy [22]. Cranial MRI showed bilateral cystic leukodystrophy with thalamic involvement extending from frontal to occipital lobes. These patients with onset of NKH in infancy died before 2 years of age; their neuropathology was somewhat similar to vanishing white matter with a cavitated leukoclastic encephalopathy, involving both hemispheres with preserved U-fibres and vacuolated demyelination involving the corpus callosum, cerebellar peduncules, medial lemniscus and pyramidal tracts [21, 23]. One 2-year-old child with normal development experienced acute deterioration progressing to spastic diplegia, with raised plasma glycine and low enzymatic activity although the plasma/CSF ratio was normal [24]. One patient developed autistic features following neonatal seizures and was shown to have serotonin metabolism defect [25]. One patient with late-onset nonketotic hyperglycinaemia sustained a successful pregnancy, and the neuropsychometric values of her offspring were average [26]. One 66-year-old woman developed mental deterioration at school age and gait disturbance with dysarthria and bradykinesia in her forties. MRI showed hypoplasia of the corpus callosum with cerebral and cerebellar atrophy. Elevated values of glycine were found in blood, CSF and urine [27].

24.2 Metabolic Derangement

The glycine cleavage system (GCS), a mitochondrial enzyme complex, is made up of four individual constituents. These are a P-protein (pyridoxal phosphate-dependent glycine decarboxylase, GLDC), a T-protein (tetrahydrofolate-requiring aminomethyltransferase, AMT), an H-protein (glycine cleavage system hydrogen carrier protein, GCSH, containing lipoic acid), and an L-protein, lipoamide dehydrogenase. These four specific proteins allow the degradation of glycine in the liver, kidney and brain (◘ Fig. 24.1). From the study of a large number of patients, it seems that most patients with the neonatal form of the disease have a very low GCS activity and that late-onset patients have some residual activity. The overall activ-

ity measured in vitro is usually, but not always, lower in P-protein deficiency than in T-protein deficiency. The mechanism for transient NKH is unclear, because of the lack of enzymatic data. Immaturity of one or more of the components of the GCS or deficiency of any of its cofactors is postulated. Since 2000, the glycine-CO_2 exchange reaction, a new assay performed on tissue obtained from liver biopsy, has allowed identification of the deficient protein [28]. About 75% of patients with NKH have a defect in the P-protein, and the remainder have a defect in the T-protein. L- or H-protein deficiencies are apparently very rare. Initial classification into probable P- or T-protein defects allows a rational search for mutations in the appropriate gene.

The pathophysiological mechanism(s) of glycine encephalopathy remain(s) obscure. They may be related to the role of glycine as an adjuvant to the NMDA receptor, which plays a major role in ontogenesis of the brain cortex (for review see [29]): the GCS is abundant in rat neuronal stem cells in the neuroepithelium [30].

Glycine also plays a major role in the developing brain neuronal excitability [31] and could account for the myoclonic epileptic encephalopathy. Indeed, the three other presently identified conditions that produce neonatal myoclonus with suppression burst are also likely to activate the NMDA receptor by causing an increase of glutamate concentration in the synaptic cleft: pyridoxine and pyridoxal phosphate deficiencies that prevent the transformation of glutamate into gamma aminobutyric acid (GABA) and therefore increase the level of glutamate, and the glutamate transporter defect [32] (► Chapter 29). In experimental models, a slight increase in glutamate when GABA is still excitatory has devastating consequences, with the development of a pattern similar to neonatal myoclonic encephalopathy [33].

In addition, glycine severely impairs brain bioenergetics at the level of energy formation, transfer and utilisation, which could contribute to neurological troubles [34].

In the spinal cord, glycine is the major inhibitory neurotransmitter leading to hypotonia [35].

24.3 Genetics

NKH is transmitted as an autosomal recessive trait. The *AMT*, *GLDC* and *GCSH* genes have been cloned [36, 37]. In the *AMT* gene, three recurrent mutations, IVS 7-1G>A, R320H and 296H, are found in about 10% of the deficient alleles. Approximately 30 other *AMT* mutations have been identified in single cases [38]. About 50 different mutations of the *GLDC* gene have been collected to date by Applegarth [38], including large deletions and missense, nonsense, splicing site and frame shift mutations. Most of these are private [39]. In Finland the majority of patients carry a S564I mutation [40]. Only R515S, T269M and A389V mutations were found in a few alleles from patients tested in Europe and Canada; all others are private. Only one mutation in the *GCSH* gene has been reported [41]. Patients in whom no mutation or only one mutation has been found, despite sequencing of the P-, T- and H-protein genes, have been reported [42]. In one series, 22.5% of cases were due to deletion within *GLDC* [43]. Available data suggest that in nonconsanguineous families the patient is likely to be a compound heterozygote. Since most reported mutations seem to be rare or private, phenotype could not be predicted from genotype. A high residual activity of the GCS is associated with the A802V mutation [14] with 32% of wild type activity or heterozygosity [15].

24.4 Diagnostic Tests

When NKH is suspected clinically, plasma amino acids should be analysed in the absence of valproate treatment. If an isolated elevation of glycine is found (control values 125-320 μmol/l), an organic acidaemia with ketotic hyperglycinaemia (most commonly propionic or methylmalonic acidaemia) must be excluded by urinary organic acid and/or plasma acylcarnitine analysis. If no abnormal metabolites are found, glycine levels should then be measured simultaneously in plasma and in CSF (provided the CSF sample is nontraumatic, since cells would increase glycine values; control values <10 μmol/l). In NKH all other amino acids are unremarkable, remaining within normal values. The diagnosis of NKH is based on the finding of either an increased absolute value of glycine in CSF or an increased CSF-to-plasma glycine ratio (control values <0.02). In classic neonatal NKH this ratio is very high (>0.08), whereas it is only slightly elevated (0.04-0.10) or even normal in late-onset, milder or atypical cases (D. Rabier, personal communication). However, no prediction of the deficient protein or of the prognosis can be derived from these values. In all cases, the diagnosis requires consistent clinical and biochemical findings. Discordant cases require enzymatic and/or molecular genetics confirmation.

In order to confirm the diagnosis by measurement of overall GCS activity and to identify the deficient protein by the glycine-CO_2 exchange reaction, a liver biopsy with about 80 mg of tissue is necessary. Neither fibroblasts nor leukocytes can be used for these assays. However, overall GCS activity is detectable in lymphoblasts ob-

tained from B-lymphocytes infected and transformed using Epstein-Barr virus [44]. When the deficient protein has been identified, sequencing of the coding region and the intron/exon boundaries can be performed on the appropriate gene.

The identification of carriers is unreliable when attempted by enzymatic analysis in lymphoblasts, and can only be done by molecular genetic testing once the mutations have been identified in the proband. NKH is usually not detectable by tandem mass spectrometry on newborn screening [45].

24.5 Differential Diagnosis

Burst suppression (BS) is not specific to NKH. When occurring with myoclonus it mostly remains undiagnosed although there is familial recurrence in over 10% of cases [46]. BS was first reported as an acute event in ischaemic encephalopathy [47, 48] and may be difficult to distinguish from fragmented hypsarrhythmia when it occurs only in sleep [49]. The combination of neonatal seizures with BS is fairly frequent and its aetiology is variable, ranging from inborn errors of metabolism to malformations [50]. The presence of fragmentary and erratic myoclonus is a major component of metabolic causes of BS, but it may be missing, as can be the case sometimes in glycine encephalopathy. The suppression component of BS with metabolic disease is often particularly long, up to 20 s [50].

BS is also a frequent finding in pyridoxine dependency and part of the clinical pattern of pyridox(am)ine phosphate oxidase deficiency, and of glutamate transporter deficiency (► Chapter 29). Both pyridoxine dependency and pyridox(am)ine phosphate oxidase deficiency may be associated with a transient rise in CSF glycine [51], which may lead to diagnostic confusion with NKH (► Chapter 29). On the other hand, BS has occasionally been described in rare cases of D-glyceric, methylmalonic and propionic acidaemias, sulfite and xanthine oxidase deficiencies, carbamoyl phosphate synthase deficiency with hyperammonaemia, and Menkes disease [50, 52-54]. Malformations associated with BS include Aicardi malformation, schizencephaly, porencephaly, hemimegalencephaly and olivodentate dysplasia [50, 55]. However, these are most often associated with tonic seizures rather than myoclonus.

On MRI, a particular leukodystrophy called vanishing white matter may be confused with NKH, since in both conditions glycine may be increased to similar values [56]. Diffusion sequence shows low apparent diffusion quotient values [57].

24.6 Prenatal Diagnosis

Since no effective treatment is available for NKH, prenatal diagnosis is frequently requested. GCS activity is present in fresh chorionic villi, but not in amniocytes or chorionic villi in culture. More than 500 prenatal diagnoses have been performed by measuring GCS activity on crude chorionic villi tissue. False, unexplained negative results have been reported in about 1% of cases [58], with mutations in *AMT* or *GLDC* genes found subsequently in four cases. Moreover, there is a range that is not amenable to interpretation, where low normal control values and affected fetuses with residual activity overlap [59]. Molecular diagnosis can now be offered. This analysis is performed on DNA extracted from fetal cells obtained by either amniocentesis (14-16 weeks) or chorionic villus sampling (10-12 weeks). If only one mutation is known, the affected gene may be identified and linkage analysis using linkage markers on the chromosome can be undertaken. Measurement of amniotic fluid glycine concentration and the glycine/serine ratio is unreliable because control values and those recorded in affected patients overlap [60].

24.7 Treatment

Treatment with sodium benzoate is usually ineffective [10], except possibly in rare transient cases [14]. Pantothenic acid administration has been proposed, because it is the precursor of coenzyme A that activates sodium benzoate [61]. In one late-onset case, a combination of a low-protein diet, sodium benzoate and imipramine was reported to have been effective [62].

The probable contribution of NMDA receptor activation has promoted therapeutic trials with compounds that reduce NMDA transmission, such as dextrometorphan, tryptophan and ketamine [63]. However, they have proved disappointing, probably because the epileptic encephalopathy begins long before birth. Valproate and vigabatrin may worsen the condition [22, 64].

Partial control of convulsive seizures was achieved with vagal nerve stimulation [65] in two cases.

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Disorders of Proline and Serine Metabolism

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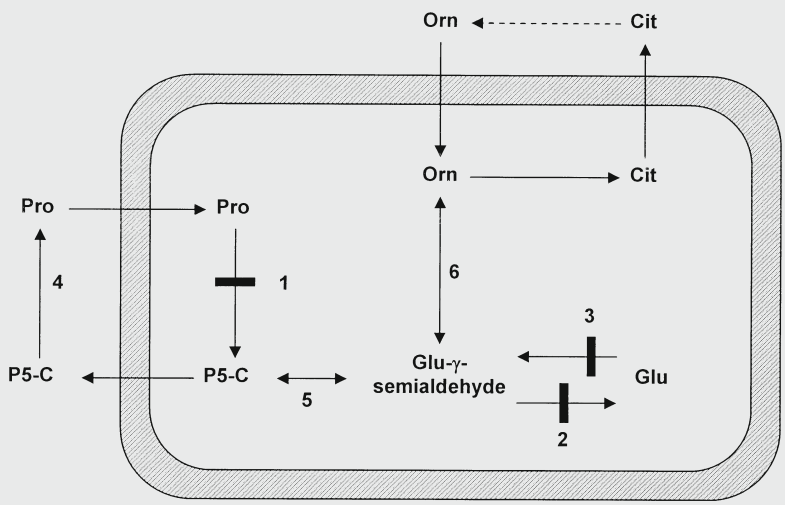
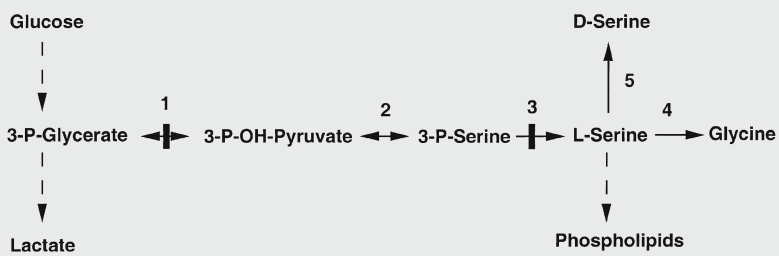
Proline and Serine Metabolism

Proline and serine are non-essential amino acids. Unlike all other amino acids (except hydroxyproline), **proline** has no primary amino group (it is termed an imino acid) and uses, as a consequence, a specific system of enzymes for its metabolism (◘ Fig. 25.1). Δ^1-Pyrroline 5-carboxylate (P5-C) is both the immediate precursor and the degradation product of proline. The P5-C/proline cycle transfers reducing/oxidising potential between cellular organelles. Owing to its pyrrolidine ring, proline (together with hydroxyproline) contributes to the structural stability of proteins, particularly collagen, with its high proline and hydroxyproline content.

Serine also has important functions besides its role in protein synthesis. It is a precursor of a number of compounds (partly illustrated in ◘ Fig. 25.2), including D-serine, glycine, cysteine, serine phospholipids, sphingomyelins and cerebrosides. 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 synthesised de novo from a glycolytic intermediate, 3-phosphoglycerate, and can also be synthesised from glycine by reversal of the reaction catalysed by serine hydroxymethyltransferase, which thereby converts N^5,N^{10}-methylene-THF to THF (◘ Fig. 24.1).



◘ **Fig. 25.1.** Proline metabolism. *Shaded area* represents mitochondrial membrane. *Cit,* citrulline; *Glu,* glutamine; *Orn,* ornithine; *Pro,* proline; *P5-C,* Δ^1-pyrroline 5-carboxylate. **1**, Proline oxidase (deficient in hyperprolinaemia type 1); **2**, P5-C dehydrogenase (deficient in hyperprolinaemia type 2); **3**, P5-C synthase (deficient in P5-C synthase deficiency) **4**, P5-C reductase (deficient in P5-C reductase deficiency) **5**, nonenzymatic reaction; **6**, ornithine aminotransferase (deficient in gyrate atrophy). *Bars* across *arrows* indicate defects of proline metabolism



◘ **Fig. 25.2.** Pathway of de novo serine synthesis. *P,* Phosphate. **1**, 3-phosphoglycerate dehydrogenase; **2**, 3-phosphohydroxypyruvate transaminase; **3**, 3-phosphoserine phosphatase; **4**, serine hydroxymethyltransferase (utilises tetrahydrofolate); **5**, serine racemase. Glycine is synthesised from serine, but also from other sources. *Bars* across *arrows* indicate the known defects in serine synthesis

Four disorders of **proline** metabolism are known: two in its catabolism (hyperprolinaemia type I, which is due to proline oxidase deficiency, and hyperprolinaemia type II, which is due to Δ^1-pyrroline 5-carboxylate dehydrogenase deficiency) and two in its synthesis (Δ1-pyrroline 5-carboxylate synthase deficiency and Δ^1-pyrroline 5-carboxylate reductase deficiency). Hyperprolinaemia type I is generally considered a nondisease, while hyperprolinaemia type II appears to be associated with a disposition to recurrent seizures. The deficiency of the proline-synthesising enzyme, Δ^1-pyrroline 5-carboxylate synthase, which is also involved in ornithine synthesis, is described in ► Chapter 22.

Five disorders of **serine** metabolism are known. Three are in its biosynthesis: 3-phosphoglycerate dehydrogenase deficiency, phosphoserine aminotransferase deficiency and phosphoserine phosphatase deficiency. Patients with 3-phosphoglycerate dehydrogenase deficiency have congenital microcephaly, psychomotor retardation and intractable seizures and are partially responsive to L-serine or L-serine and glycine. One patient with an association of Williams syndrome and phosphoserine phosphatase deficiency has been reported. Another, unexplained, serine disorder has been reported in a patient with decreased serine in body fluids, ichthyosis and polyneuropathy but no central nervous system manifestations. There was a spectacular response to L-serine. Recently, hereditary sensory neuropathy type 1 has been found to be associated with mutations of serine palmitoyltransferase, the initial step in the **de novo** synthesis of sphingolipids.

25.1 Inborn Errors of Proline Metabolism

25.1.1 Proline Oxidase Deficiency (Hyperprolinaemia Type I)

- **Clinical Presentation**

Hyperprolinaemia type I is a very rare disorder; it is generally considered a benign trait, but recent work suggests that it may be associated with a subset of schizophrenic patients [1-4].

- **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 P5-C (◘ Fig. 25.1, enzyme 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 have hyperprolinaemia.

- **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. At least 16 missense mutations have been identified [4, 5].

- **Diagnostic Tests**

The diagnosis is made by amino acid analysis. Direct enzyme assay is not possible, since the enzyme is not present in leukocytes or skin fibroblasts. Mutation analysis is thus necessary to confirm the diagnosis [4].

- **Treatment and Prognosis**

Since the prognosis is generally excellent, dietary treatment is not indicated.

25.1.2 Δ^1-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 [2].

- **Metabolic Derangement**

Hyperprolinaemia type II is caused by a deficiency of pyrroline 5-carboxylate (P5-C) dehydrogenase, a mitochondrial inner-membrane enzyme involved in the conversion of proline into glutamate (◘ Fig. 25.1, enzyme 2). 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 P5-C. Heterozygotes do not have hyperprolinaemia. Evidence has been presented that the accumulating P5-C is a vitamin B_6 antagonist (owing due to adduct formation) and that the seizures in this disorder may be due at least in part to vitamin B_6 inactivation [6, 7].

- **Genetics**

This is an autosomal recessive disease. The gene *ALDH4A1* maps to 1p36. Mutations have recently been reported in four patients (two frame shift mutations and two missense mutations) [8].

- **Diagnostic Tests**

The accumulation of P5-C in physiological fluids is used to differentiate between type II and type I hyperproli-

naemia. This compound can be qualitatively identified by its reactivity with ortho-aminobenzaldehyde and can be quantitatively measured by several specific assays [2]. P5-C 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.

25.1.3 Δ^1-Pyrroline 5-Carboxylate Reductase Deficiency

▪ Clinical Presentation

Forty patients from 24 families have been reported [9, 10]. They showed a cutis laxa type 2 syndrome comprising intrauterine growth retardation, progeroid appearance (wrinkly loose skin most prominent over the dorsum of the hands and feet, and craniofacial dysmorphy), joint laxity, psychomotor retardation, hernias, osteopenia, and less consistent features such as cataracts and athetoid movements. This disorder resembles pyrroline 5-carboxylate synthase deficiency, the other disorder of the proline synthesis pathway, but the latter disorder shows a more severe neurological phenotype.

25.2 Inborn Errors of Serine Metabolism

25.2.1 3-Phosphoglycerate Dehydrogenase Deficiency

▪ Clinical Presentation

At least nine patients belonging to four families are known with this disease, which was first reported in 1996 [11, 12]. They presented at birth with microcephaly and developed pronounced psychomotor retardation, severe spastic tetraplegia, nystagmus and intractable seizures (including hypsarrhythmia).

In addition, one patient showed congenital bilateral cataract, two siblings, growth retardation and hypogonadism, and two other siblings, megaloblastic anaemia. Magnetic resonance imaging of the brain revealed cortical and subcortical atrophy and evidence of disturbed myelination.

Recently, a much milder phenotype has been reported in two siblings [13]. Absence seizures occurred after the ages of 5 and 9 years, and there was moderate psychomotor retardation without microcephaly.

▪ Metabolic Derangement

The deficiency of 3-phosphoglycerate dehydrogenase, the first step of serine biosynthesis (◘ Fig. 25.2, enzyme 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 anaemia, decreased methyltetrahydrofolate was found in CSF. This can be explained by the fact that serine is converted to glycine by a reaction that forms methylenetetrahydrofolate (◘ Fig. 24.1), which is further reduced to methyltetrahydrofolate (► Chapter 28).

▪ Genetics

This is an autosomal recessive disease. The gene for 3-phosphoglycerate dehydrogenase has been mapped to 1q12. Several mutations have been identified [12, 14]. Prenatal diagnosis is only possible by mutation analysis, as there is a lack of data on enzyme activity in chorionic villi and amniocytes [15].

▪ Diagnostic Tests

The diagnosis should be suspected in patients with encephalopathy who have congenital microcephaly. Plasma amino acids must be measured in the fasting state (range for 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, albeit to a lesser extent. The diagnosis is confirmed by finding deficient activity of 3-phosphoglycerate dehydrogenase in fibroblasts (reported residual activities of 6-22%). In patients with the milder juvenile phenotype, the metabolite and enzymatic findings were indistinguishable from those in patients with the severe phenotype [13].

▪ Treatment and Prognosis

Treatment with L-serine has a beneficial effect on the convulsions, spasticity, feeding and behaviour. Oral L-serine treatment (up to 600 mg/kg/day in six divided doses) corrected the biochemical abnormalities in all reported patients and abolished the convulsions in most patients, 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 [16]. In two patients, convulsions stopped only after glycine (200 mg/kg/day) was added.

In a girl diagnosed prenatally, because of decelerating head growth, L-serine was given to the mother at 190 mg/kg/day in three divided doses from week 27 of gestation. This normalised fetal head growth, and with subsequent postnatal therapy the girl showed normal psychomotor development at the age of 3 years [15].

Patients with the milder phenotype have been diagnosed as teenagers and responded favourably to low dosages of L-serine therapy [13].

25.2.2 Phosphoserine Aminotransferase Deficiency

This disorder has been reported in a brother and sister, who showed decreased concentrations of serine and glycine in plasma and CSF [17]. 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 measured in cultured fibroblasts was inconclusive, but mutation analysis revealed compound heterozygosity in *PSAT1* in both children.

25.2.3 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 one patient with Williams syndrome [18]. Phosphoserine phosphatase activity in lymphoblasts and fibroblasts amounted to about 25% of normal (◘ Fig. 25.2, enzyme 3). Oral serine normalised 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 [19].

25.2.4 Serine Deficiency with Ichthyosis and Polyneuropathy

A remarkable new serine deficiency syndrome has been discovered by De Klerk et al. [20] in a 15-year-old girl. She had ichthyosis from the 1st year of life and growth retardation from the age of 6 years, and presented at the age of 14 years with walking difficulties and areflexia, symptoms of an axonal polyneuropathy. Psychomotor development and magnetic resonance imaging of the brain were normal. Fasting plasma and CSF serine levels were decreased, but the CSF glycine level slightly increased. Oral ingestion of serine (400 mg/kg/day) cured the skin lesions and the polyneuropathy. It is hypothesised that this patient exhibits increased conversion of serine into glycine, possibly owing to hyperactivity of serine hydroxymethyltransferase (◘ Fig. 25.2, enzyme 4).

25.2.5 Serine Palmitoyltransferase Defects

They cause the most frequent subtype of hereditary sensory and autonomic neuropathy, HSAN type 1 (HSAN1), an autosomal dominant disease. The disorder has been shown to be caused by mutations in the *SPTLC* genes, encoding three subunits of serine palmitoyltransferase (SPT), the first step in the de novo synthesis of sphingolipids (► Chapter 37).

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Transport Defects of Amino Acids at the Cell Membrane: Cystinuria, Lysinuric Protein Intolerance and Hartnup Disorder

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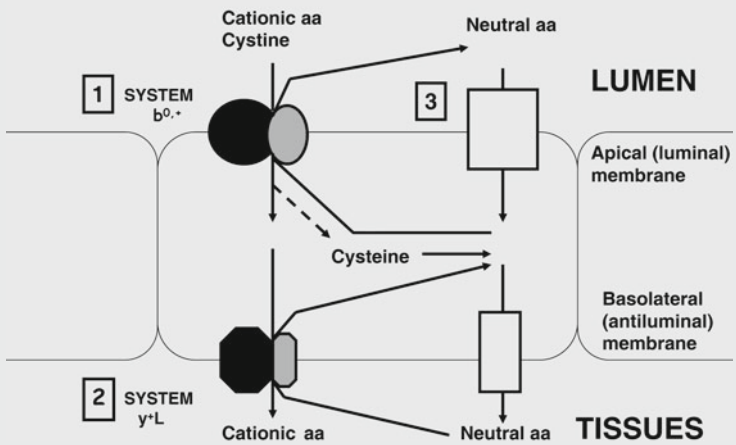
Transepithelial Transport of Amino Acids

Epithelial cells in (for example) renal tubules and intestinal mucosa utilise several different amino acid transport systems (◘ Fig. 26.1), which prefer amino acids with certain physicochemical properties. Cystine and the structurally related dibasic amino acids lysine, arginine and ornithine are transported from the intestinal or renal tubular lumen into the epithelial cells by an *apical transporter* ([1]: *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* ([2]: *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 colocalise in the plasma membrane, but the exact process of dimerisation 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* [3] is expressed only at the luminal border of the epithelial cells. It transports alanine, asparagine, citrulline, glutamine, histidine, isoleucine, leucine, phenylalanine, serine, threonine, tryptophan, tyrosine and valine into the epithelial cells. A specific renal transporter for the imino acids, glycine, proline and hydroxyproline, and the dicarboxylic amino acids, aspartate and glutamate, probably exists.

Cystinuria, lysinuric protein intolerance and Hartnup disorder are caused by defects of the *luminal cystine/dibasic amino acid transporter* [1], the *antiluminal dibasic amino acid transporter* [2], and the *neutral amino acid transporter* [3], respectively. The mechanism of imino and dicarboxylic aminoaciduria is still unknown.



◘ **Fig. 26.1.** Simplified schematic representation of cationic and neutral amino acid transport in epithelial cells. *aa*, amino acids. (Modified from [1])

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 of certain amino acids in urine or lack of them in 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. Deficiencies of the two intermediates of the urea cycle lead to protein intolerance, and lack of lysine probably contributes to growth retardation and skeletal and immunological manifestations. The pellagra-like dermatitis and ataxia in Hartnup disorder are attributed to deficiency of tryptophan, the precursor of niacin synthesis.

26.1 Cystinuria

26.1.1 Clinical Presentation

Cystinuria is linked with a life-long risk of urolithiasis [2]. It is responsible for 1-2% of kidney stones in adults and 6-8% in children. Occasional 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 [3]. Recurrent urinary tract infections, urinary obstruction and, finally, renal insufficiency are possible complications. Cystine stones are usually radioopaque and also visible on ultrasonography.

With increasing knowledge of the genetics of cystinuria, its classification is changing from phenotypic (based on urinary amino acid excretion in obligate heterozygotes) to mutation based. Cystinuria associated with severe neurological findings or Prader-Willi-like syndrome suggests a contiguous gene deletion on chromosome 2p16 [4, 5] (▶ Section 26.1.3).

26.1.2 Metabolic Derangement

In cystinuria, the high-affinity luminal transporter (system $b^{0,+}$; ◘ Fig. 26.1) for cystine and dibasic amino acids in the epithelial cells of the proximal renal tubulus and in jejunal mucosa is defective. The defect leads to poor absorption of cystine in the intestine and its poor reabsorption in the kidney. Whereas normally 99% of the filtered cystine is reabsorbed, homozygotes with cystinuria excrete 600-1,400 mg of cystine per day and crystals and stones may be formed. No signs of cystine deficiency have been described, however.

26.1.3 Genetics

The average incidence of cystinuria is 1 in 7,000 but varies considerably between different populations. Cystinuria can be classified into two subtypes: type I cystinuria, the pure autosomal recessive form of the disease that represents over 60% of the cases, and non-type 1 cystinuria, which is inherited in a dominant mode with incomplete penetrance. As obligate heterozygote carriers, the parents of type I patients have a normal urinary amino acid profile, while the cystine excretion of the parents of non-type 1 patients ranges from normal to clearly elevated.

Type I cystinuria is caused by mutations in the *SLC3A1* gene on chromosome 2p16. The gene encodes the heavy subunit of the amino acid transporter, rBAT. More than 125 mutations in the *SLC3A1* gene have been reported. Non-type I cystinuria results from mutations in the *SLC7A9* gene on chromosome 19q13.11. The gene product is the light subunit of the transporter, $b^{0,+AT}$. Over 50 mutations have been described. A patient may also have inherited a type 1 allele from one parent and a non-type I allele from the other. Most of the mutations of these two genes have been detected only in single patients, and the distribution of the more frequent ones is associated with ethnic background [6-8]. A genetics-based classification for cystinuria has been suggested: type A for *SLC3A1* homozygotes (corresponding to type I cystinuria), type B for *SLC7A9* homozygotes (most of which represent non-type I cystinuria), and type AB for the mixed type [9, 10]. While SLC3A1 and SLC7A9 mutations including unbalanced genomic rearrangements explain most of the cases, it is possible that other genes may be involved [11]. A recessive contiguous gene deletion of chromosome 2p16 associated with cystinuria and mitochondrial disease has been described in a large kindred [4] and another, named hypotonia-cystinuria syndrome, mimicking Prader-Willi syndrome, is due to mutation in the *PREPL* gene [5].

26.1.4 Diagnostic Tests

A positive urinary nitroprusside test and analysis of urinary amino acids lead to the diagnosis. Homozygotes with cystinuria excrete more than 0.1 mmol cystine/mmol creatinine (250 mg cystine/g creatinine) into the urine, but the excretion varies markedly. Plasma concentrations of cystine and the dibasic amino acids are normal or slightly decreased. 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.

26.1.5 Treatment and Prognosis

Excessive hydration to dilute the urine and alkalinisation to improve cystine solubility are the cornerstones of therapy. Moderate sodium restriction is recommended, as reduced sodium intake decreases cystine excretion. Adults should consume more than 3,000 ml fluid/24 h (1.75–2 l/m^2/24 h), 500 ml of this before bedtime and, if possible, 500 ml during the night. Because cystine is much more soluble in alkaline urine (500 mg/l at pH 7.5 vs about 250 mg/l at pH 7.0), permanent alkalinisation of urine is beneficial. Sodium bicarbonate (1.5-2 mmol/kg/day in four doses) has commonly been used, but in patients with normal renal function, potassium citrate (in equal doses) would probably be preferable as it does not increase the sodium load. Restriction of dietary animal protein in order to reduce methionine intake and thus limit endogenous cystine synthesis may be helpful [12, 13].

If the standard therapy fails to prevent new or dissolve pre-existing stones, a thiol derivative is added to decrease urinary free cystine concentration by forming

water-soluble disulfide compounds [14]. D-Penicillamine (2 g/1.73 m^2 body surface area/day divided in three doses; up to 3 g/day in adults) is well tolerated by most patients but may cause hypersensitivity reactions, renal problems or a variety of autoimmune syndromes. 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 [15]. Mercaptopropionylglycine (tiopronin) has fewer adverse effects, but has occasionally caused glomerulopathy or hyperlipidaemia. The dose (15-20 mg/kg/day up to 1,000 mg/day in three doses in adults;10-50 mg/kg/day in children [16]) is adjusted individually. The drug has also been successfully used (one dose every 2 days) as stone prophylaxis [17]. Acetazolamide 500 mg at bedtime has been effective in small number of adult patients in increasing urinary pH but it is relatively poorly tolerated and can induce hypokalaemia and calcium phosphate stones [18] Captopril is well tolerated but may not be as effective as thiol compounds [19].

Percutaneous nephrolithotomy and extracorporeal shock-wave lithotripsy are seldom effective in stone removal, because cystine stones are extremely hard. However, some centres use shock-wave lithotripsy as the primary treatment option in stones that have a surface area of less than 300 mm^2 [20]. New, minimally invasive urological techniques in experienced hands minimise the need for open surgery [21]. Surgical procedures should always be combined with conservative preventive therapy.

Regular follow-up is mandatory to support compliance with the treatment, to monitor renal function and to detect developing stones early. New tools for monitoring the efficacy of therapy have been developed. Determination of cystine crystal volume in morning urine, in addition to urinary pH and specific gravity [22], or direct assessment of urinary supersaturation [23] may prove helpful. Early detection of the disease by screening the family members of a patient is also essential. Homozygotes with type I cystinuria frequently develop stones during the 1st decade of life and should perhaps be treated prophylactically. Other subtypes may have a milder course [24]. Renal function is frequently impaired as a result of stone-forming cystinuria.

26.2 Lysinuric Protein Intolerance

26.2.1 Clinical Presentation

Over 130 patients with LPI, over 50 of them from Finland, have been reported or are known to us. The full natural history of LPI still remains to be characterised, as the oldest patients are still in their sixties. Newborns and infants are usually asymptomatic if they are only fed breast milk. Postprandial episodes of hyperammonaemia usually emerge when the infants begin to receive formula with higher protein content, or supplementary high-protein foods [25, 26]. Hyperammonaemia may present as refusal to eat, vomiting, stupor and unconsciousness. 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 are moderately enlarged.

In toddlers and school-age children, the presenting signs are most often growth failure and hepato- and splenomegaly. The children are usually hypotonic, muscular endurance is decreased, and they 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 marked delay of puberty.

The clinical heterogeneity of LPI is obvious in adult patients. Most 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 skeletal changes, e.g., osteopenia [27, 28], but pathological fractures seldom occur. Radiological signs of pulmonary fibrosis are common, but few patients suffer from symptomatic interstitial lung disease [29]. Mental capacity varies from normal to moderate retardation, depending on any 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 [30], while serum iron and transferrin concentrations are normal. Most adult patients have combined hyperlipidaemia [31]. High serum immunoglobulin-G concentrations and abnormalities in the distribution of lymphocyte subpopulations as well as in humoral immune responses [32, 33] have been reported. Varicella infections are usually severe and can be fatal [34]. Several cases of systemic lupus erythematosus have been reported [35-37]. Bone marrow involvement with hemophagocytic lymphohistiocytosis and interstitial pulmonary disease with alveolar proteinosis are also quite frequent complications, and can occasionally be presenting signs [38-43].

Disturbed tubular function with mild proteinuria, glucosuria, phosphaturia, and tubular acidosis, microscopic haematuria and slow decrease of glomerular filtration rate have been reported in several patients. Systematic screening has revealed renal dysfunction of variable degree in about every other Finnish patient, with rapid deterioration in glomerular filtration in some cases [44].

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 [43]. One child with alveolar proteinosis went through a successful heart-lung transplantation, but died later after a recurrent disease [45].

Pregnancies of patients with LPI have been complicated by toxaemia, anaemia or bleeding during the delivery and variable degrees of intrauterine growth retardation, but many have been completed successfully without any major problems [46].

26.2.2 Metabolic Derangement

In LPI, transport of the dibasic cationic amino acids lysine, arginine and ornithine (system y^+L; ◘ Fig. 26.1) is defective at the basolateral membrane of epithelial cells in the renal tubules and small intestine [47, 48].

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 the malfunction of the urea cycle. It is still unclear whether the transport defect is also expressed in nonepithelial cells. Contrary to an earlier report [49], more recent data indicate that fibroblasts from LPI patients have normal cationic amino acid transport, probably via other transporter isoforms [50]. Also, erythrocytes possess intact cationic amino acid transport [51, 52]. A transport defect in hepatocytes has been postulated because of normal or elevated cationic amino acid concentrations in liver biopsy in LPI, and the possibility of abnormal cationic amino acid transport between various intracellular compartments has been suggested [53].

Hyperammonaemia after protein ingestion and diminished protein tolerance in LPI resemble the symptoms of urea cycle enzyme deficiencies. The malfunction of the urea cycle in LPI is best explained by functional deficiency of the intermediates arginine and ornithine in the hepatocytes [53]. Most patients develop a protective aversion to high-protein foods, which further impairs their amino acid intake, aggravating the amino acid deficiencies and protein nutrition. As arginine is the rate-limiting precursor of nitric oxide synthesis, arginine deficiency may also result in persistently low nitric oxide concentrations that may influence vascular and immunological functions [54, 55]. Reduced availability of lysine, an essential amino acid, probably has a prominent role in the poor growth and skeletal and immunological manifestations of the patients. Occasional patients have severe carnitine deficiency [56-58] 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 pathogenetic mechanisms of several clinical manifestations of LPI are still unknown.

26.2.3 Genetics

LPI is a rare autosomal-recessive disease, with fewer than 200 patients reported. The incidence is highest in Finland (1 in 60,000); clusters of families are also known in Italy, Norway and Japan, and sporadic cases have been reported on all continents. The gene *SLC7A7* on chromosome 14q encodes the light subunit of the dibasic amino acid transporter y^+LAT-1. At least 43 different mutations spread along the entire gene have been reported [57, 59-62].

All Finnish patients share the same founder mutation, 1181-2A>T, which causes a frame shift and 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.

26.2.4 Diagnostic Tests

The diagnosis of 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 or an intravenous L-alanine load. Orotic aciduria is practically always seen postprandially in untreated patients. Nonspecific but consistent findings in LPI patients include elevated serum lactate dehydrogenase activity and increased ferritin and zinc concentrations.

In the genetically homogenous Finnish population, the diagnosis is easily confirmed by mutation analysis.

26.2.5 Treatment and Prognosis

The principal aims of treatment are to prevent hyperammonaemia and to provide a sufficient supply of protein and essential amino acids for normal metabolism and

growth. The protein tolerance in LPI can be improved with supplementary citrulline, a neutral amino acid that is also an intermediate in the urea cycle. Citrulline is readily absorbed and partially converted to arginine and ornithine. All the three amino acids improve the function of the urea cycle. Approximately 100 mg/kg/day of L-citrulline is given in three to five doses in association with protein-containing meals [63]. On citrulline supplementation, children usually tolerate 1.0-1.5 g/kg/day and adults 0.5-0.8 g/kg/day of protein without hyperammonaemia or increased orotic acid excretion [64]. There is marked interindividual variation in the protein tolerance, and infections, pregnancy and lactation may alter it extensively. Frequent monitoring of urinary orotic acid excretion is necessary for optimal therapy. For patients with constantly highly elevated glutamine and glycine levels, we have added sodium benzoate or sodium phenylbutyrate (both up to 250 mg/kg/day or 13 g/m^2/day) to diminish the nitrogen load of the urea cycle.

Correction of lysine deficiency in LPI is complicated by its poor intestinal absorption and the resulting osmotic diarrhoea [65]. However, a carefully titrated dose of L-lysine-HCl is able to elevate the plasma lysine concentrations to low-normal range without gastrointestinal or other side effects. We currently supplement all our patients with 20-30 mg/kg/day of the compound in three doses [66, 67].

Carnitine supplementation (1 g/day) is indicated for the patients with carnitine deficiency [58]. Owing to their restricted diet, the patients need regular supplementation with calcium, vitamins and trace elements, and the involvement of an experienced nutritionist is essential. We have recently started growth hormone therapy in four children with growth retardation, with a promising early response and no side effects (unpublished data). Similarly, we have treated elevated serum cholesterol values successfully with statin therapy [68].

The rare cases of acute hyperammonaemia in LPI patients should be treated as in urea cycle defects (► Chapter 20). All protein- and nitrogen-containing substances are removed from the nutrition, and sufficient energy is supplied as intravenous glucose. An infusion of arginine or citrulline improves the function of urea cycle, and sodium benzoate and/or sodium phenylbutyrate utilise alternate pathways of ammonia elimination [69].

LPI patients should be immunised against Varicella zoster, and nonimmunised patients should be treated immediately with aciclovir if they get the infection [34]. The treatment of the immunological and bone marrow complications, including haemophagocytic lymphohistiocytosis, is still experimental. Good responses have been reported in individual cases with immunosuppressive drugs and with immunoglobulin infusion [70]. In alveolar proteinosis, bronchoalveolar lavage and steroid therapy have been effective in some cases [38-41]. Granulocyte-macrophage colony-stimulating factor therapy is probably not suitable in LPI [43, 71]. One child has received a heart-lung transplant, but alveolar proteinosis recurred in the transplanted lungs [45].

Although hyperammonaemia and the associated mental retardation can be avoided with citrulline therapy, several other complications of LPI, especially renal involvement, seem to develop and progress during current therapy. The accumulating knowledge of many multisystem manifestations of LPI is challenging the previous concept that it is, in most cases, a fairly benign and easily treated condition [72].

26.3 Hartnup Disorder

26.3.1 Clinical Presentation

The classic clinical symptoms of Hartnup disorder, pellagra-like dermatitis and neurological involvement, 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 [73], an extensive number of subjects who fulfil the biochemical diagnostic criteria have been reported, mostly detected in newborn screening (NBS) 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 [74] and tend to ameliorate with increasing 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, and the skin becomes scaly and rough with peripheral hypopigmentation. Eruptions may mimic those seen in zinc deficiency. Intermittent cerebellar ataxia, attacks of headache, muscle pain and weakness may appear. Occasional patients present with mental retardation, seizures or psychiatric symptoms. Growth and developmental outcome of the patients are generally normal, although also low academic scores have been reported.

26.3.2 Metabolic Derangement

The pattern of hyperaminoaciduria in Hartnup disease is characteristic. Alanine, serine, threonine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, histidine and citrulline and the monoamino-dicarboxylic

amides asparagine and glutamine are excreted in 5- to 20-fold excess in the urine, and their plasma concentrations are decreased or low normal. Renal clearance values of other amino acids are within the normal range. The molecular defect involves a sodium-dependent and chloride-independent neutral amino acid transporter, SLC6A19, shared by the affected neutral monoamino-monocarboxylic amino acids and expressed predominantly in the epithelial cells in the renal proximal tubuli and intestinal epithelium [75, 76] but also in skin [77]. The stools of the patients contain increased amounts of free amino acids, closely reflecting the urinary excretion pattern [78]. After an oral tryptophan load, the patients show smaller plasma tryptophan peaks than controls and excrete smaller amounts of tryptophan metabolites in the urine. The affected amino acids are readily absorbed as short oligopeptides but not as free amino acids. 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.

The clinical manifestations that resemble niacin deficiency probably reflect deficient production of the tryptophan metabolite nicotinamide. Symptomatic disease may thus be prevented if dietary niacin intake is sufficient, or if the necessary amount of tryptophan is absorbed in oligopeptide form [79].

26.3.3 Genetics

Hartnup disorder follows an autosomal recessive pattern of inheritance. The reported incidence in newborns screened for aminoaciduria has varied from 1 in 14,000 to 1 in 45,000. Hartnup disorder is caused by mutations in the gene encoding the neutral amino acid transporter B(0)AT1 (*SLC6A19*) on chromosome 5p15.33 [75, 76, 80, 81]. Two mutated SLC6A19 alleles, whether identical or not, are necessary for the manifestation of characteristic aminoaciduria. To date, at least 21 mutations have been identified, of which D173N seems to be the most common.

26.3.4 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.

26.3.5 Treatment and Prognosis

Dermatitis and neurological symptoms usually but not invariably disappear rapidly with oral nicotinamide (50-300 mg/day). An adequate supply of high-quality protein is probably important for prevention of the symptoms. 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 characterised. Early recognition of the condition in NBS programmes permits adequate follow-up and prevention of symptomatic disease. Maternal Hartnup disorder seems to be harmless to the fetus [82].

26.4 Asymptomatic Aminoacidurias

Screening programmes for urinary amino acids have detected asymptomatic patients with iminoglycinuria and dicarboxylic aminoaciduria. In iminoglycinuria increased excretion of glycine, proline and hydroxyproline is detected in the urine [83, 84]. As iminoglycinuria is normal in newborns, reflecting probably renal immaturity, the finding needs to be confirmed later. The incidence of iminoglycinuria is 1 in 10,000.

Iminoglycinuria is inherited in an autosomal recessive mode. Interestingly, the parents of the homozygous individuals (obligate heterozygotes) show glycinuria only. The molecular cause of iminoglycinuria is not known at present, but candidate genes include *SLC36A1* and *SLC6A20*, encoding a proton-dependent amino acid transporter, PAT-1, and a sodium-dependent imino acid transporter, SIT-1, and *SLC6A19* encoding the transporter B^0AT1. Although iminoglysinuria has occasionally been linked to other diseases in case reports, the present view is that it does not lead to any clinical symptoms in spite of constant urinary loss of the three amino acids.

Dicarboxylic aminoaciduria, i.e. excess urinary excretion of the acidic amino acids (aspartate and glutamate), is another asymptomatic and probably largely benign condition that has been detected in screening programmes. The estimated incidence of dicarboxylic aminoaciduria is 1 in 35,000. An acidic amino acid transporter EAAC1/EAAT3, encoded by the gene *SLC1A1*, is an obvious candidate for the molecular mechanism; however, no mutations of the gene have so far been detected.

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V Vitamin-Responsive Disorders

Biotin-responsive Disorders

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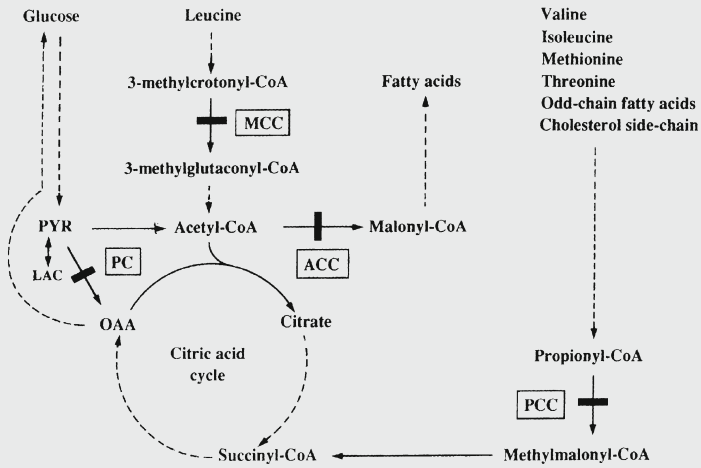
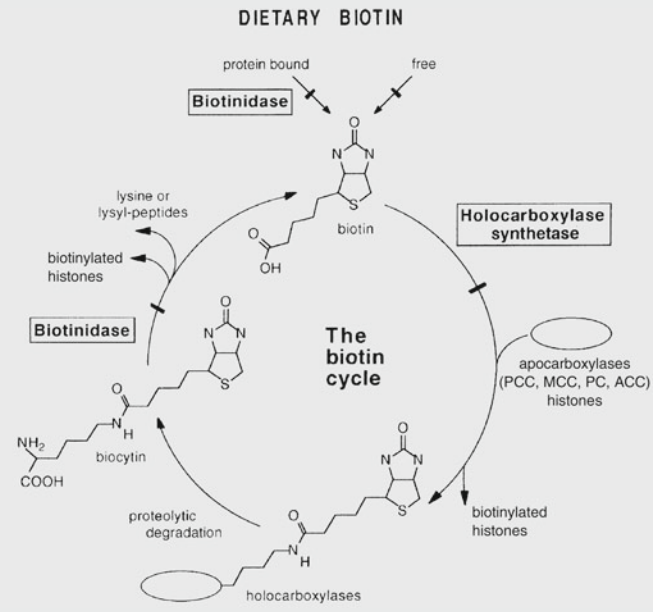
The Biotin Cycle and Biotin-dependent Enzymes

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 four important carboxylases involved in gluconeogenesis, fatty acid synthesis and the catabolism of several amino acids (◘ Fig. 27.1). Covalent binding of biotin to the four inactive apocarboxylases catalysed by *holocarboxylase synthetase* (HCS) is required to generate the active holocarboxylases (◘ Fig. 27.2). 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. 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 [1]. In addition, both HCS and biotinidase bind biotin covalently to histones. Thus biotin modifies the structure of chromatin playing a role in cell proliferation, gene silencing and DNA repair [2].



◘ **Fig. 27.1.** Location of the biotin-dependent carboxylases in intermediary metabolism. *ACC*, acetyl-CoA carboxylase; *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. 27.2.** The biotin cycle. Abbreviations as in Fig. 27.1. 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. A defect in *biotin transport* has been reported in a single child; however the genetic defect remains unresolved to date. *Biotin-responsive basal ganglia disease* is an autosomal recessive disorder with childhood onset that presents with subacute episodes of encephalopathy often triggered by febrile illness which disappears within a few days without neurological sequelae if biotin is administered early.

27.1 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, gene symbol *HLCS*) and biotinidase deficiency [3]. 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, necessitating confirmation of the diagnosis by enzyme assay.

27.1.1 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 [4, 5]. 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 [6, 7].

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 last 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 [8].

27.1.2 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 [9]. 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. Skin rash and/or alopecia are hallmarks of the disease; however, these may develop late or not at all [10, 11]. 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 kera-

toconjunctivitis. 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 [12]. Their symptoms are usually less characteristic and may include motor limb weakness, spastic paraparesis, spinal cord demyelination and unusual symmetrical findings on brain MRI [13, 14], or eye problems such as loss of visual acuity and scotomata [12].

Asymptomatic adults and siblings with profound biotinidase deficiency, some with very low 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 [15-18]. 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 [14, 19-21]. Late-diagnosed patients often have psychomotor retardation and neurological symptoms, such as leukoencephalopathy, hearing loss and optic atrophy, which may be irreversible [10, 11, 14, 20-23]. Outcome may even be fatal. One patient died at the age of 22 months, with features of Leigh syndrome confirmed by histopathology [19].

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 [24]. 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 [19] 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, 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 [20, 22]. 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 [9].

27.1.3 Biotin-responsive Basal Ganglia Disease

Biotin-responsive basal ganglia disease (BBGD) is an autosomal recessive disorder with childhood onset that presents with subacute episodes of encephalopathy, often triggered by febrile illness and characterised by confusion, dysarthria and dysphagia, that progresses to severe cogwheel rigidity, dystonia, quadriparesis and, if left untreated, to permanent dystonia or even coma and death [25]. Administration of high doses of biotin during encephalopathic crises resulted in partial or complete recovery within days. Brain MRI displayed characteristic bilateral lesions of the caudate nucleus and putamen. Most patients diagnosed to date are of Saudi, Syrian or Yemeni ancestry, but a report of a patient of European ancestry has recently been published, suggesting that BBGD is a panethnic condition [26].

27.2 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 four holocarboxylases from their corresponding inactive apocarboxylases at physiological biotin concentrations (◘ Fig. 27.2) [27-29]. In biotinidase deficiency, biotin cannot be released from biocytin and short biotinyl peptides. Thus, patients with biotinidase deficiency are unable to either recycle endogenous biotin or to use protein-bound dietary biotin (◘ Fig. 27.2) [3]. Consequently, biotin is lost in the urine, mainly in the form of biocytin [9, 30], 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 carboxylases in both HCS and biotinidase deficiencies (◘ Fig. 27.1) results in accumulation of lactic acid and derivatives of 3-methylcrotonyl-coenzyme A (CoA) and propionyl-CoA (► Section 27.4).

Isolated inherited deficiencies of each of the three mitochondrial carboxylases, propionyl-CoA carboxylase (PCC), 3-methylcrotonyl-CoA carboxylase (MCC); (for both, ► Chapter 19), and pyruvate carboxylase (PC; ► Chapter 12), are also known. A single patient with an isolated defect of acetyl-CoA carboxylase (ACC, cytosolic) has been reported [31]. 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 recently been reported [32].

In BBGD there is a defective cerebral transport of thiamin via the biotin-sensitive transporter hTHTR2 [33, 34].

Acquired biotin deficiency is rare, but may result from excessive consumption of raw egg white, 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 and nutritional biotin intake [35], but the genetic defect remains unresolved to date.

27.3 Genetics

Both HCS and biotinidase deficiency are inherited as autosomal recessive traits. HCS deficiency seems to be rarer than biotinidase deficiency. 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 [36]. The incidence of combined profound and partial deficiency is about 1 in 60,000. The cDNAs for human HCS [37, 38] and biotinidase [39] have been cloned, and the corresponding genes have been mapped to human chromosomes 21q22.1 [38] and 3p25 [40], respectively. In both genes, multiple disease causing mutations have been identified.

27.3.1 Holocarboxylase Synthetase Deficiency

More than 35 different disease-causing mutations have been reported in the *HLCS* gene [41-45]. About two-thirds of them are within the putative biotin-binding region of HCS and result in decreased affinity of the enzyme for biotin [27, 29, 41, 43, 46]; this probably accounts for the in vivo responsiveness to biotin therapy of these patients. The degree of abnormality of the K_m values of HCS for biotin correlates well with the time of onset and severity of illness, i.e. highest K_m with early onset and severe disease [28].

Other mutations, located outside the biotin-binding site in the N-terminal region, are associated with normal K_m but decreased V_{max} [29]. 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 [47]. 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 [41]. This mutation leads to reduced half-life of HCS and seems to be prevalent in Polynesian patients of Samoan origin [48].

27.3.2 Biotinidase Deficiency

Mutation analysis of the entire biotinidase gene is rarely necessary, because there are no therapeutic consequences, except in individuals with partial biotinidase deficiency, in whom targeted mutation analysis may be helpful [49]. Over 100 different mutations have been identified in patients with profound or partial biotinidase deficiency [44, 50, 51]. The two most common mutations detected in symptomatic patients with profound deficiency in the USA, accounting for about one-third of the alleles, are c.98-104del7ins3 and p.R538C [50, 52]. 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 [50]. Strikingly, these mutations were not detected in any of the symptomatic patients [50, 52]. Furthermore, none of the symptomatic children had detectable serum biotinidase biotinyl-transferase activity, while two-thirds of the children identified by screening had detectable activity [53].

A comparison of all mutations identified in children detected by newborn screening and in symptomatic children revealed four mutations comprising 59% of all mutant alleles [54]. Only two of these four mutations occurred in both populations. Thus, it is possible that individuals with certain mutations in the newborn screening group may have a decreased risk of developing symptoms.

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 [49].

27.3.3 Biotin-responsive Basal Ganglia Disease

BBGD is an autosomal recessive disorder which has been associated with mutations in the *SLC19A3* gene mapping to human chromosome region 2q36.3 and encoding the thiamine transporter hTHTR2 [33]. Biotin is not a substrate for this transporter. However, biotin levels have been shown to regulate the expression of *SLC19A3* [55], and rescue of the clinical phenotype through high doses of biotin is likely to be the result of increased expression

of the receptor leading to restoration of some function. Interestingly, mutations in the same *SLC19A3* gene cause a Wernicke's-like encephalopathy, which is responsive to thiamine [56].

27.4 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.

There is no metabolic marker in BBGD.

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 [11].

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 [5, 17]. 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.

27.4.1 Holocarboxylase Synthetase Deficiency

- Biotin concentrations in plasma and urine are normal.
- Carboxylase activities in lymphocytes are deficient and cannot be activated by in vitro preincubation with biotin [3].
- Direct measurement of HCS activity requires a protein, e.g. an apocarboxylase or an apocarboxyl carrier protein of ACC, as one of the substrates [28, 57]; therefore, it is not routinely performed.
- HCS deficiency can be diagnosed indirectly by demonstrating severely decreased carboxylase activities in fibroblasts cultured in a medium with low biotin concentration (10^{-10} mol/l) and by normalisation (or at least an increase) of the activities in cells cultured in media supplemented with high biotin concentrations (10^{-6}-10^{-5} mol/l) [5, 28]. 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, which results in a final biotin concentration of about 10^{-8} mol/l [5, 7].

27.4.2 Biotinidase Deficiency

- Biotinidase activity in plasma is absent or decreased [17, 36]. Many patients have measurable residual activity and should be evaluated for the presence of a K_m defect (► below).
- Symptomatic patients usually have decreased biotin concentrations in plasma and urine [9, 17], provided that an assay method that does not detect biocytin is used [58]. In addition, carboxylase activities in lymphocytes are usually decreased, but are normalised within hours after either a single dose of oral biotin [9] or in vitro preincubation with biotin [3, 17].
- Patients excrete biocytin in urine [30], the concentration being dependent on the level of residual biotinidase activity [17].
- 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 medium.

27.4.3 Acquired Biotin Deficiency

- Biotinidase activity is normal in plasma.
- Biotin concentrations are low in plasma and urine.
- Carboxylase activities in lymphocytes are decreased and are promptly normalised after a single dose of oral biotin or after preincubation with biotin in vitro [3].

27.4.4 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 enzymatic investigation in these cases [7]. Prenatal diagnosis allows rational prenatal therapy, preventing severe metabolic derangement in the early neonatal period [7, 59]. Biotinidase can be measured in chorionic villi or cultured amniotic fluid cells but, in our opinion, this is not warranted, because prenatal treatment is not necessary.

27.5 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. No adverse effects have been observed from such therapy over a more than 20-year experience of treating biotinidase deficiency [49] and, importantly, there is no accumulation of biocytin in body fluids [30], which was previously suspected to be a possible risk.

Restriction of protein intake is not necessary except in very severe cases of HCS deficiency. Acutely ill patients with metabolic decompensation require general emergency treatment in addition to biotin therapy (► Chapter 4).

27.5.1 Holocarboxylase Synthetase Deficiency

The required dose of biotin is dependent on the severity of the enzyme defect and has to be assessed individually [3]. 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 [3, 5, 59-62]. 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 activity in lymphocytes. Exceptionally, persistent clinical and biochemical abnormalities have been observed despite treatment with very high doses of biotin [3, 41, 59-61]. All patients with HCS deficiency have at least partially responded to pharmacological doses of biotin with the exception of those homozygous for the missense mutation p.L216R [41].

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 [3, 41, 59, 60, 61]. 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 [63]. In five Japanese patients (four families), the intelligence quotient (IQ) at the age of 5-10 years varied between 64 and 80 [59]. Four of these patients had a severe neonatal-onset form, and one of them (IQ=64) was treated prenatally. Three of these patients 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 [7, 59]. It is unclear whether prenatal treatment is essential; treatment of at-risk children immediately after birth may be sufficient.

27.5.2 Biotinidase Deficiency

The introduction of neonatal screening programmes has resulted in the detection of asymptomatic patients with residual biotinidase activity [36]. Based on measurement of plasma biotinidase activity, the patients are classified into three main groups.

- Patients with profound biotinidase deficiency, with less than 10% of mean normal serum biotinidase activity.
- Patients with partial biotinidase deficiency, with 10-30% residual activity.
- Patients with decreased affinity of biotinidase for biocytin, i.e. K_m variants [64].

▪ Group 1

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 [9].

Neonatal screening for biotinidase deficiency [36] 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 [9].

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 [10, 11, 22-24], and intellectual impairment and ataxia have been observed as long-term complications [10, 20, 22, 23].

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 [15-17]. 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 with (e.g.) 2.5 mg of biotin per day [17, 36, 49].

- **Group 2**

Patients with partial biotinidase deficiency (10-30% residual activity) are mostly detected by neonatal screening and in family studies and usually remain asymptomatic. One infant with about 30% enzyme activity developed hypotonia, skin rash and hair loss during an episode of gastroenteritis at 6 months of age. This was reversed by biotin therapy [65]. We showed that among 24 patients with 14-25% serum biotinidase activity studied at the age of 8 months to 8 years, 16 patients had a subnormal biotin concentration in at least one plasma sample, with a tendency toward lower values with increasing age [66]. 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 small doses of biotin, e.g. 1-5 mg/day [49].

- **Group 3**

Among 201 patients (176 families), we found ten patients (eight families) with a K_m defect. Six patients (five families) showed profound deficiency (0.9-3% residual activity in routine assay conditions), whereas four patients (three families) showed partial deficiency (18-20% residual activity). The index patient in all five families with profound deficiency presented with a severe clinical illness [21, 64], and one of the patients with partial deficiency, although apparently asymptomatic, had marginal biotin deficiency at the age of 2 years [64]. Thus, all patients with a K_m defect seem to have a high risk of becoming biotin deficient and require biotin therapy. However, because most laboratories do not perform detection of K_m variants, and because increasing number of patients with partial biotinidase deficiency have been reported to develop biotin-responsive symptoms at some time during their life [49], it may be safer to treat also all patients with partial biotinidase deficiency with biotin.

Elevated serum biotinidase activities have been reported in most patients with hepatic glycogen storage disease [67]. The reason for this finding is still unknown, but biotinidase has proved to be a useful biomarker for these disorders.

27.5.3 Biotin-responsive Basal Ganglia Disease

All clinical symptoms of BBGD disappear within a few days with the administration of high doses of biotin (5-10 mg/kg/day) if the patient is treated early. They reappear within 1 month if biotin is discontinued. Patients who are diagnosed late or have had repeated episodes suffer from residual symptoms, such as paraparesis, mild mental retardation or dystonia [25]. One sibling of a patient of European origin did not improve when treated with high-dose biotin alone, but markedly and rapidly improved when thiamine was added to biotin [26].

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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. 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, it is a cofactor of the cytoplasmic enzyme methionine synthase. As adenosylcobalamin, 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.

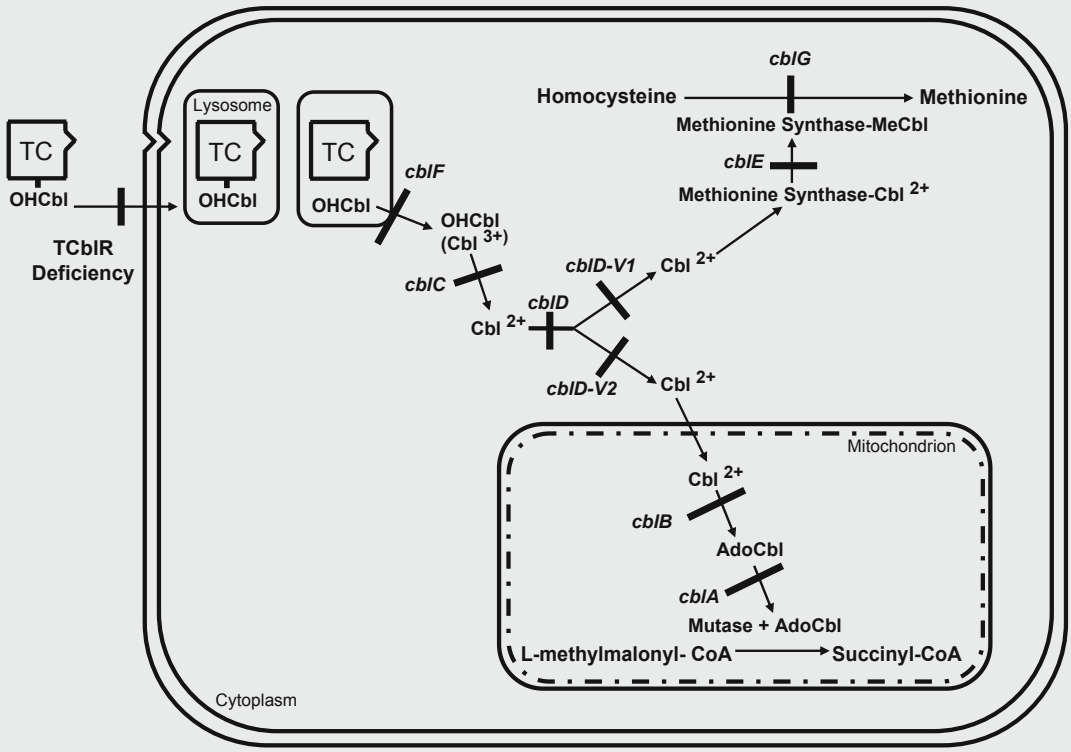


Fig. 28.1. Cobalamin (*Cbl*) endocytosis and intracellular metabolism. The cytoplasmic, lysosomal, and mitochondrial compartments are indicated: *AdoCbl*, adenosylcobalamin; *CoA*, coenzyme A; *MeCbl*, methylcobalamin; *OHCbl*, hydroxocobalamin; *TC*, transcobalamin (previously TCII); *V1*, variant 1; *V2*, variant 2; $\mathbf{1}^{+}$, $\mathbf{2}^{+}$, $\mathbf{3}^{+}$ refer to the oxidation state of the central cobalt of Cbl; *cblA*-*cblG* refer to the sites of blocks. »Mutase + AdoCbl« refers to the active form of methylmalonyl-CoA mutase, which contains a molecule AdoCbl. Enzyme defects are indicated by *solid bars*

The serum cobalamin (Cbl) level is usually low in patients with disorders affecting absorption and transport of Cbl, with the exception of transcobalamin (TC) deficiency. Patients with disorders of intracellular Cbl metabolism typically have normal serum Cbl levels, although levels may be reduced in the *cblF* disorder. Homocystinuria and hyperhomocysteinaemia, as well as megaloblastic anaemia and neurological disorders, are major clinical findings in patients with disorders of cobalamin absorption and transport, as well as those with defects of cellular metabolism that affect synthesis of methylcobalamin (MeCbl). Methylmalonic aciduria and acidaemia, resulting in metabolic acidosis, are seen in disorders that result in decreased synthesis of adenosylcobalamin (AdoCbl).

Inherited disorders of cobalamin metabolism are divided into those involving absorption and transport and those involving intracellular utilisation [1-4].

28.1 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 a specific receptor called 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.

28.1.1 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 [5-7]. 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 nonfunctional. There have been reports of IF with reduced affinity for Cbl or cubam, or with increased susceptibility to proteolysis [6-8].

▪ Genetics

Fewer than 50 patients of both sexes have been reported. Inheritance is autosomal recessive. A 4-bp deletion (c.183_186delGAAT) in the coding region of the gastric IF (*GIF*) gene on chromosome 11q13 [9] was identified as the cause of IF deficiency in an 11-year-old girl with severe anaemia and Cbl deficiency [10]. A number of additional *GIF* mutations have been identified in patients with IF deficiency [11,12].

▪ 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. A deoxyuridine suppression test on marrow cells can be useful but is no longer available in most clinical laboratories. In hereditary IF deficiency, in contrast to acquired forms of pernicious anaemia, there is normal gastric acidity and normal gastric cytology. Cbl absorption, as measured by the Schilling test, is abnormal, but is normalised when the labelled Cbl is mixed with a source of normal IF, such as gastric juice from an unaffected individual. 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 relevant genes may represent an appropriate first-line means of correctly diagnosing these disorders [11].

▪ 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 normal values may be as low as 0.25 mg every 3 months. If treatment is delayed, some neurological abnormalities may persist in spite of complete reversal of the haematological and biochemical findings.

28.1.2 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 fetal 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 [13-20]. Most patients have proteinuria that is not of the classic glomerular or tubular types, does not respond to therapy with cobalamin and is not progressive [14, 21]. 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 cobalamin therapy [22]. 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, which comprises two components. Cubilin was first purified as the IF-Cbl receptor from the proximal renal

tubule [23-25]. A second component, amnionless, colocalises with cubilin in the endocytic apparatus of polarised epithelial cells, forming a tightly bound complex that is essential for endocytic function [26]. Thus defective function of either protein may cause this disorder.

▪ Genetics

Over 300 cases have been reported. Inheritance is autosomal recessive [20], with environmental factors affecting expression [24, 27]. Most patients are found in Finland, Norway, Saudi Arabia and Turkey, and among Sephardic Jews. The cubilin gene *(CUBN)* has been mapped to 10p12.1. A P1297L mutation was found in 31 of 34 disease chromosomes from 16 of 17 Finnish families segregating megaloblastic anaemia [28]. Linkage studies in families from Norway without mutations of the *CUBN* gene led to the discovery of the amnionless gene *(AMN)*. A study of 42 MGA1 sibships confirmed *CUBN* mutations in Finnish and *AMN* mutations in Norwegian patients. Mutations of both *CUBN* and *AMN* have been identified in patients of Eastern Mediterranean origin. Evidence was also provided for a possible additional Imerslund-Gräsbeck syndrome-causing gene locus [29, 30].

▪ Diagnostic Tests

In contrast to patients with IF deficiency, the Schilling test is not corrected by providing a source of human IF with the labelled Cbl [1]. 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 [11].

▪ 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.

28.1.3 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 [31] 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 cobalamin levels, and this has also been identified in individuals with serum levels within the reference range [32-34].

▪ Metabolic Derangement

The role of haptocorrin is uncertain, but it could be involved in the scavenging of toxic Cbl analogues or in protecting circulating methylcobalamin from photolysis [35, 36]. Deficiency of haptocorrin has been described in isolation and in association with deficiency of other specific granule proteins such as lactoferrin [37].

▪ Genetics

The haptocorrin gene has been cloned and mapped to chromosome 11q11-q12 [38, 39]. A patient with severe deficiency of haptocorrin has been shown to be compound heterozygous for two nonsense mutations in the *TCN1* gene (c.270delG and c.315C→T). 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 [34].

▪ 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.

28.1.4 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 fetal 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. Leukaemia may be mistakenly diagnosed

because of the presence of immature white cell precursors in an otherwise hypocellular marrow [40]. 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 [41]. Neurological features include developmental delay, neuropathy, myelopathy and encephalopathy and, rarely, retinal degeneration [42]. Defective granulocyte function with both defective humoral and cellular immunity may occur.

▪ Metabolic Derangement

The majority of patients have no immunologically detectable TC, although others have some detectable TC that is able to bind Cbl but lacks normal function [1, 43, 44].

▪ Genetics

Inheritance is autosomal recessive. There have been at least 40 cases, including both twins and siblings [1, 41]. Disease-causing deletions, nonsense mutations and activation of an intra exonic cryptic splice site, as well as a number of polymorphic variants, have been described in the *TCN2* gene on chromosome 22q11.2-qter [45-47].

▪ 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. Since TC is involved in the transcytosis of Cbl through the enterocyte, the Schilling test may be abnormal in TC-deficient patients. In those patients in whom the Schilling test is normal, immunoreactive TC is found. Reports of levels of Cbl-related metabolites are scarce and inconsistent. For example, normal plasma total homocysteine and moderately increased urine methylmalonic acid was reported in three patients and methylmalonic aciduria and homocystinuria, without specified levels, in one patient [42,48].

Study of TC synthesis in cultured fibroblasts or amniocytes allows both pre- and postnatal diagnosis in patients who do not synthesise TC [49]. 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 [50].

▪ 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 the rapid loss of vitamin 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.

28.1.5 Transcobalamin Receptor Deficiency

▪ Clinical Presentation

Several patients 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. Patients had moderate elevations of serum methylmalonic acid and, in most cases, of homocysteine, but otherwise did not show clinical signs of cobalamin deficiency [51, 52].

▪ Metabolic Derangement

Uptake of TC-bound radioactive cobalamin was decreased in cultured fibroblasts from all of the patients, 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 patients with this disorder are homozygous or heterozygous for a 3-bp deletion (c.262_264delGAG) in the *CD320* gene on chromosome 19p13.2 that encodes the transcobalamin receptor. This mutation has been shown to diminish cobalamin uptake in an in vitro system [51]. A second mutation, c.297delA, was present in the heterozygous state in two patients [52]. The 3-bp deletion was present at a frequency of 3% in an Irish control population [53].

▪ Treatment and Prognosis

Since none of the patients have clinical signs of cobalamin deficiency, it is likely that treatment is not necessary.

28.2 Disorders of Intracellular Utilisation of Cobalamin

A number of disorders of intracellular metabolism of Cbl have been classified as *cbl* mutants (*A-G*), based on the biochemical phenotype and on genetic complementation analysis (◘ Fig. 28.1).

28.2.1 Combined Deficiencies of Adenosylcobalamin and Methylcobalamin

Three distinct disorders are associated with functional defects in both methylmalonyl-coenzyme A (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 smallness for gestational age, 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 [54-57].

▪▪ Metabolic Derangement

The defect in *cblF* appears to be a failure of Cbl transport across the lysosomal membrane following degradation of TC in the lysosome. As a result, Cbl cannot be converted to either adenosylcobalamin (AdoCbl) or methylcobalamin (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

Thirteen patients with the *cblF* disorder have been reported. Mutations in the *LMBRD1* gene on chromosome 6q13 have been shown to underlie the disorder [56]. This gene encodes a lysosomal membrane protein that is presumed to function in transport of free cobalamin across the lysosomal membrane into the cytoplasm. All except two of the patients have been homozygous or heterozygous for a deletion (c.1056delG) which is found on a common haplotype [56, 58].

▪▪ Diagnostic Tests

The serum Cbl level may be low, and the Schilling test has been abnormal in all patients tested. Usually, increased plasma total homocysteine, 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. Precise diagnosis of the inborn errors of Cbl metabolism requires tests in cultured fibroblasts. The incorporation of [^{14}C]propionate into macromolecules is a good screen for the integrity of the methylmalonyl-CoA mutase reaction, and the incorporation of [^{14}C]methyltetrahydrofolate or the conversion of labelled formate to methionine reliably measures the function of methionine synthase. Measurement of the total incorporation of [^{57}Co]CNCbl by fibroblasts and its conversion to both MeCbl and AdoCbl can differentiate a number of the disorders. 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 incorporation of both labelled propionate and labelled methyltetrahydrofolate.

▪▪ 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-C

▪▪ Clinical Presentation

This is the most frequent inborn error of Cbl metabolism, and several hundred patients are known [59-63]. 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 nonregenerative 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 [64-68]. Congenital structural heart defects may be present [69]. 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 [64, 70-73]. The patients in this group who were diagnosed earlier had findings overlapping those found in the

younger onset group. Major clinical findings in this 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. 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 mutations in the *MMACHC* gene, which encodes a protein that plays a role in the early steps of cellular cobalamin metabolism [60]. The MMACHC protein binds cobalamin and appears to catalyse removal of upper axial ligands from alkylcobalamins (including the methyl group from MeCbl and the adenosyl group from AdoCbl) and from CNCbl [74-76].

■■ Genetics

The *MMACHC* gene responsible for *cblC* is on chromosome 1p23.2 [60]. A common mutation, c.271dupA, accounts for 40% or more of all disease alleles in patient populations of European origin [60-62]. A different mutation, c.609G→A (p.W203X), represents over 50% of disease-causing alleles in Chinese *cblC* patients [63]. Inheritance is autosomal recessive. Prenatal diagnosis can be performed by mutation analysis or by measuring the incorporation of labelled propionate or labelled methyltetrahydrofolate and the synthesis of MeCbl and AdoCbl 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 [77].

■■ Diagnostic Tests

Increased plasma total homocysteine, low to normal plasma methionine, homocystinuria and methylmalonic 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 incorporation of label from propionate, methyltetrahydrofolate (or formate) and CNCbl, and there is decreased synthesis of both AdoCbl and MeCbl. Cells fail to complement those of other *cblC* patients.

■■ Treatment and Prognosis

Treatment with 1 mg/day OHCbl (parenteral) decreases the elevated metabolite levels, but these are not usually completely normalised. In one comprehensive study, oral OHCbl was found to be insufficient and both folinic acid and carnitine were ineffective. Daily oral betaine (250 mg/kg/day) with twice-weekly systemic OHCbl (1 mg/day) resulted in normalisation of methionine and homocysteine levels and decreased methylmalonic aciduria [78]. Another study found that as much as 20 mg OHCbl a day was necessary to correct methylmalonate and homocysteine levels in one patient [79]. Even though oral administration of OHCbl generally appears not to be effective, this route was reported to be successful in one patient [80]. Both in vitro studies and studies of patients indicate that CNCbl is less effective than OHCbl in treatment of this disease [81, 82], possibly reflecting the role of the MMACHC protein in decyanation of CNCbl [74].

Of a group of 44 patients with onset in the 1st year of life, 13 died and only 1 patient was neurologically intact, with other survivors described as having severe or moderate impairment. Survival with mild to moderate disability was found in the patients who had a later onset [59].

■ Cobalamin-D

■■ Clinical Presentation

This defect was first described in two brothers [83-85]. 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 [86]. Following the discovery of the *cblD* gene [87] further patients were described. We now know of a total of 21 cblD patients, 6 with isolated homocystinuria, 9 with isolated methylmalonic aciduria and 6 with combined methylmalonic aciduria/homocystinuria. [87, 88].

■■ Metabolic Derangement

The *cblD* defect is caused by mutations in the *MMADHC* gene and can cause deficient synthesis of both AdoCbl and MeCbl together, or of either in isolation. This suggests that either the product of *MMADHC* is a multifunctional protein, or that there are least two different products of *MMADHC*, which each play a role in directing cobalamin from the MMACHC protein to the two cobalamin-dependent enzymes.

■■ Genetics

The *MMADHC* gene responsible for *cblD* is located on chromosome 2q23.2 and codes for a protein of 32.8 kD. Biallelic 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 a second start codon [87].

■■ 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* 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 analysis to make a specific diagnosis in the *cbl* defects.

28.2.2 Adenosylcobalamin Deficiency

■ Clinical Presentation

Adenosylcobalamin (AdoCbl) deficiency comprises *cblA* and *cblB*, two disorders characterised by methylmalonic aciduria (MMA) which is often Cbl-responsive [2]. The phenotype resembles methylmalonyl-CoA mutase deficiency (► Chapter 19). Most patients have an acidotic crisis in the 1st year of life, many in the neonatal period. Symptoms are related to methylmalonic acid accumulation and include vomiting, dehydration, tachypnoea, lethargy, failure to thrive, developmental retardation, hypotonia and encephalopathy. The toxic levels of methylmalonic acid may result in bone marrow abnormalities and produce anaemia, leukopenia and thrombocytopenia. Hyperammonaemia, hyperglycinaemia and ketonuria may be found.

■ Metabolic Derangement

The defect in *cblB* is deficiency of cobalamin adenosyltransferase, which catalyses the final step in intramitochondrial synthesis of AdoCbl, the cofactor for methylmalonyl-CoA mutase [89, 90]. The defect in *cblA* results from mutations in the *MMAA* gene [91]. 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 [92, 93].

■ Genetics

The *MMAA* gene has been localised to chromosome 4q31.1-q31.2 [91]. It encodes a polypeptide that belongs to the G3E family of GTP-binding proteins. Many mutations in the *MMAA* gene have now been described among *cblA* patients [91, 94-97].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.

The *MMAB* gene on chromosome 12q24 encodes cobalamin adenosyltransferase. A number of mutations in *MMAB* have been identified in *cblB* patients [90, 96, 98]. Virtually all of these mutations are clustered in the regions of the protein identified as the active site of adenosyltransferase [99].

■ Diagnostic Tests

Total serum Cbl is usually normal. Urinary methylmalonic acid levels are elevated above control values (typically <5 μmol/mmol creatinine), sometimes to greater than 20,000 μmol/mmol creatinine [100,101], but there is no increase of plasma total homocysteine or homocystinuria. A decrease in the level of methylmalonic acid excretion in response to Cbl therapy is useful in distinguishing these disorders from methylmalonyl-CoA-mutase deficiency, although many *cblB* patients do not respond to therapy with cobalamin. There has been marked variation in the form and dosage of cobalamin 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 [101].

The exact differentiation of *cblA* and *cblB* from mutase deficiency depends on fibroblast studies or sequencing of *MMAA* and *MMAB*. In both *cblA* and *cblB* levels of methylmalonyl-CoA mutase are normal in the presence of added AdoCbl. The incorporation of labelled propionate is decreased in both *cblA* and *cblB* and is usually responsive to the addition of OHCbl to the culture medium. Uptake of labelled CNCbl is normal, but there is decreased synthesis of AdoCbl. Adenosyltransferase activity is clearly deficient in *cblB*, but normal in *cblA* fibroblast extracts. Complementation 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, and it may be that AdoCbl does not reach the target enzyme intact. 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 [102, 103].

28.2.3 Methylcobalamin Deficiency

Clinical Presentation

Formation of methylcobalamin (MeCbl) is disturbed in the *cblE* and *cblG* disorders. The most common clinical findings are megaloblastic anaemia and neurological disease [104-107]. 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 [108]. Another *cblG* patient, who was diagnosed during his 4th decade of life, had mainly psychiatric symptoms. Two patients with minimal findings and without clear neurological features have also been reported [109].

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 apoenzyme. 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. The methionine synthase reductase gene, *MTRR*, has been localised to chromosome 5p15.2–15.3 [110], and a number of mutations have been identified in *cblE* patients [110-113]. The most common of these is c.903+469T→C, which represents 25% of mutant alleles. Mutations in the methionine synthase gene, *MTR*, on chromosome 1q43, have been found in *cblG* patients [114-116]. The most common of these mutations, c.3518C→T (p.1173L), represents over 40% of identified disease-causing alleles; this mutation has never been identified in the homozygous state [116]. Patients with the *cblG* variant form of methionine synthase deficiency, characterised by lack of cobalamin binding by methionine synthase protein, have null mutations [117].

Diagnostic Tests

Homocystinuria and hyperhomocysteinaemia are almost always found in the absence of methylmalonic acidaemia. However, one *cblE* patient had transient unexplained methylmalonic aciduria. Hypomethioninaemia and cystathioninaemia may be present, and there may be increased serine in the urine. Fibroblast extracts from *cblE* patients have normal activity of methionine synthase in the standard assay, but deficient activity can be found when the assay is performed under limiting reducing conditions [104]. Cell extracts from *cblG* patients have decreased methionine synthase activity in the presence of excess reducing agent. Incorporation of labelled methyltetrahydrofolate or formation of methionine from labelled formate 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, and AdoCbl is particularly increased in cblG. Complementation analysis distinguishes *cblE* from *cblG* patients.

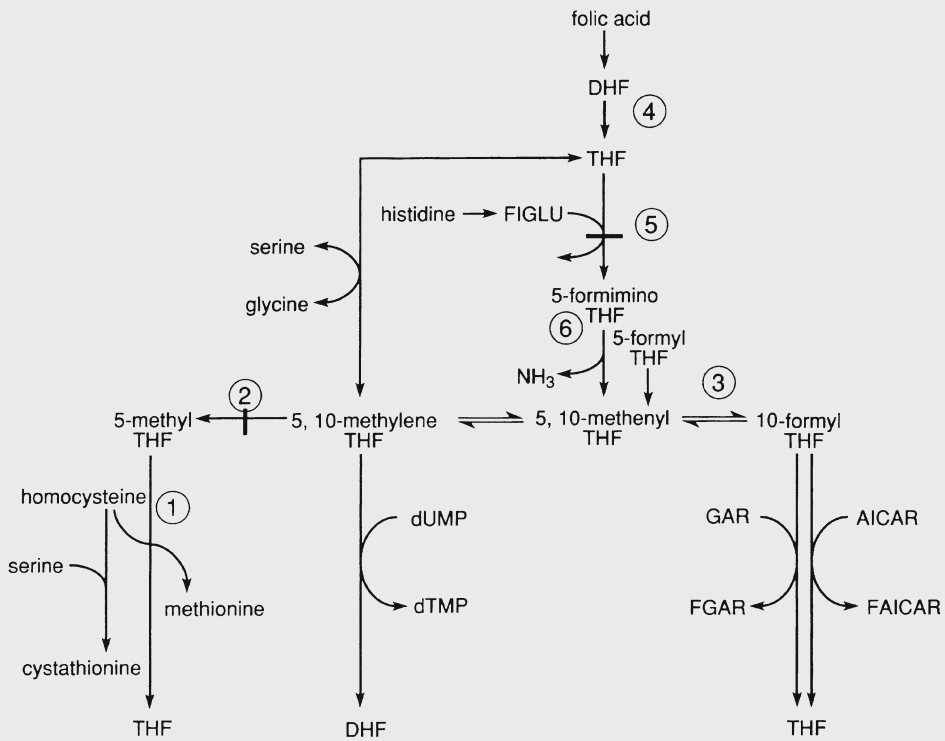
Treatment and Prognosis

Both of these 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 the neurological findings once they have developed. Treatment with betaine (250 mg/kg/day) has been used, and one *cblG* patient was treated with L-methionine (40 mg/kg/day) and had neurological improvement. Despite therapy, many patients with *cblG* and *cblE* have a poor outcome. In one family with *cblE*, there was successful prenatal diagnosis using cultured amniocytes. The mother was treated with OHCbl twice per week beginning during the 2nd trimester, and the baby was treated with OHCbl 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 his metabolic decompensation in infancy and who at 18 years old had significant developmental delay [118, 119]. Some patients may benefit from high-dose folic or folinic acid treatment.

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. 28.2). 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. The reduced folate carrier (RFC) supports a low-affinity high-capacity system for uptake of reduced folates at micromolar concentrations [120]. 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 [120]. The protein-coupled folate transporter (PCFT) supports uptake of reduced and oxidised folates at acid pH [121]. Uptake of folate in the intestine appears to depend on function of the PCFT; the RFC is expressed in enterocytes, but mutations in the gene encoding RFC are not associated with deficient intestinal folate transport, while mutations in the PCFT are. Transport of folate across the blood-brain barrier at the choroid plexus appears to require both PCFT and FRα [122].



◘ **Fig. 28.2.** Folic acid metabolism: **1**, methionine synthase; **2**, methylenetetrahydrofolate reductase; **3**, methenyltetrahydrofolate cyclohydrolase; **4**, dihydrofolate reductase; **5**, glutamate formiminotransferase; **6**, formiminotetrahydrofolate cyclodeaminase; *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*

Five confirmed inborn errors of folate absorption and metabolism have been described:

Hereditary folate malabsorption presents with severe megaloblastic anaemia, owing to the importance of dTMP and purine synthesis in haematopoiesis, and is usually associated with progressive neurological deterioration.

Cerebral folate transporter deficiency presents with psychomotor decline, progressive movement disturbance, epilepsy and profound hypomyelination in the absence of megaloblastic anaemia.

Glutamate-formiminotransferase deficiency has been reported in association with various degrees of psychomotor retardation and megaloblastic anaemia.

Severe methylenetetrahydrofolate reductase (MTHFR) deficiency presents mainly with developmental delay, often accompanied by seizures, microcephaly and findings related to cerebrovascular events. Patients typically show hyperhomocysteinaemia without megaloblastic anaemia.

Dihydrofolate reductase deficiency presents with megaloblastic anaemia, cerebral folate deficiency, seizures and, in some cases, cerebral atrophy and severe developmental delay.

28.3 Disorders of Absorption and Metabolism of Folate

28.3.1 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 [123]. Peripheral neuropathy has been seen, as have partial defects in humoral and cellular immunity.

■ 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) [121]. The haematological and gastrointestinal manifestations of this disease, but not the neurological manifestations, can be reversed by pharmacological, but relatively low, levels of folate. Folate metabolism in cultured fibroblasts is normal.

■ Genetics

Approximately 20 patients with this disorder have been reported. It is caused by mutations affecting the *SLC46A1* gene on chromosome 17q11.2, which encodes the PCFT [121]. A number of different mutations have been described, most of which have been shown to affect folate transport by the PCFT in an in vitro system.

■ 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 [124]. Folate absorption has been 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 findings but are less effective in correcting the neurological findings and in raising the level of folate in the CSF. Both methyl-THF and folinic acid may be more effective in raising CSF levels and have 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 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 [123].

28.3.2 Cerebral Folate Deficiency

■ Clinical Presentation

This disorder presents in the 1st year of life, with psychomotor retardation, spastic paraplegia, cerebellar ataxia and dyskinesia, associated with normal blood folate levels

and low folate levels only in the cerebrospinal fluid (CSF) [125, 126]. Several affected children have developed autistic features. This disorder should be differentiated from the decrease in cerebral folate levels observed in patients with acquired (perinatal asphyxia, CNS infection) and genetic (Rett syndrome, Kearn Sayre disease, MTHFR deficiency, white matter disease) disorders.

▪ 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 function at the choroid plexus.

▪ Genetics

Cerebral folate deficiency has been reported to occur as a result of antibodies directed against FRα (folate receptor α) [127]. Mutations in the *FOLR1* gene on chromosome 11q13.3-q13.5, which encodes FRα, have been identified in a small number of families [128-130]. Cerebral folate deficiency segregates as an autosomal recessive trait in families with *FOLR1* mutations.

▪ 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 [127]. Folinic acid therapy can restore CSF folate concentrations, reverse white matter choline and inositol depletion and consecutively improve clinical symptoms [128].

28.3.3 Glutamate-Formiminotransferase Deficiency

▪ Clinical Presentation

Over a dozen patients have been described, but the clinical significance of this disorder is still unclear [4, 131, 132]. 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 [133].

▪ Genetics

The human gene has been cloned and localised to chromosome 21q22.3. Hilton et al. found mutant alleles in three patients and concluded that they represent the molecular basis for this disease, although expressed residual activity was 60% [133].

▪ 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.

28.3.4 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 (► [134] for review)

▪ Clinical Presentation

Over 100 patients with severe MTHFR deficiency have been described [2, 66, 132, 135-138] or are known to the authors. 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. An infant had extreme progressive brain atrophy, and the magnetic resonance image showed demyelination [139]. A 10-year-old boy had findings compatible with those of Angelman syndrome [140]. At least one adult with severe enzyme deficiency was completely asymptomatic. Autopsy find-

ings 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 is an elevation of total plasma homocysteine levels and decreased levels of methionine. Total CSF folate levels are also 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. 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 [141].

▪ Genetics

MTHFR deficiency is inherited as an autosomal recessive disorder. There have been multiple affected children of both sexes with either unaffected parents or affected families with consanguinity. Prenatal diagnosis has been reported using amniocytes, and the enzyme is present in chorionic villi. Over 50 mutations causing severe deficiency have been described, in addition to polymorphisms that result in intermediate enzyme activity and that may contribute to disease in the general population [142-148]. Most of these mutations are restricted to one or two families. An exception is a mutation that is present at high frequency in the old order Amish [149].

▪ 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). Homocystinuria is also seen, with a mean of 130 mmol/24 h and a range of 15-667 mmol/24 h. These values are much lower than are seen in cystathionine synthase deficiency. 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. There is a rough inverse correlation between the specific activity of the reductase in cultured fibroblasts and the clinical severity. There is a better inverse correlation between clinical severity and either the proportion of total cellular folate that is in the form of methyl-THF or the extent of labelled formate incorporation into methionine. The clinical heterogeneity in MTHFR deficiency can be seen at the biochemical level. Some of the patients have residual enzyme that is more thermolabile than the control enzyme [150]. Others have been shown to have an increased K_m for NADPH [146].

▪ 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 [151-154]. 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, an enzyme that 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, cobalamin and carnitine. 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 [155].

28.3.5 Dihydrofolate Reductase Deficiency

▪ Clinical Presentation

Three families with apparent dihydrofolate reductase deficiency have been described recently [156, 157]. Findings

have included megaloblastic anaemia, cerebral folate deficiency and seizures, and in severe cases, pancytopenia, cerebral atrophy and severe developmental delay,

▪ Metabolic Derangement

While plasma and red cell folate levels are within the normal range, the forms present differ from those of controls. There are relatively high levels of oxidised 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 the *DHFR* gene on chromosome 5q11.2-q13.2 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 the *FOLR1* gene 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.

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VI Neurotransmitter and Small Peptide Disorders

Disorders of Neurotransmission

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Neurotransmitters

Classic 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) systems. The addition of neuropeptides (the largest family of signalling molecules in the nervous system) and channels (that modulate neurotransmitter actions) will probably reclassify neurotransmitter systems and disorders in the future.

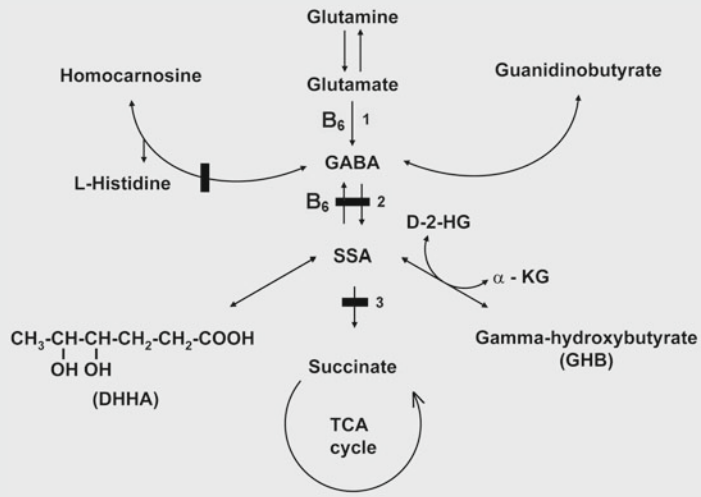
GABA is formed from glutamic acid by glutamic acid decarboxylase (◘ Fig. 29.1). It is catabolised into succinate through the sequential action of two mitochondrial enzymes, GABA transaminase and succinic semialdehyde dehydrogenase. Glutamic acid decarboxylase and GABA transaminase require pyridoxal phosphate as a coenzyme. Pyridoxal phosphate also participates in the synthesis of dopamine and serotonin (◘ Fig. 29.3), and is involved 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 grey matter. GABA modulates brain activity by binding to sodium-independent, high-affinity, mostly $GABA_A$ receptors.

Glycine, a nonessential 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.

Monoamines have a diffuse innervation in the brain and are associated with movement control, emotions, cognitive and vegetative functions.

29



◘ **Fig. 29.1.** Brain metabolism of γ-aminobutyric acid (*GABA*): B_6, pyridoxal phosphate; *DHHA*, 4,5-dihydroxyhexanoic acid; α-ketoglutarate; D-2-HG, D-2-hydroxyglutarate, *SSA*, succinic semialdehyde; *TCA*, tricarboxylic acids. **1**, glutamic acid decarboxylase; **2**, GABA transaminase; **3**, succinic semialdehyde dehydrogenase. 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. Defects of their receptors and transporters – and disorders involving vitamine B_6 (pyridoxine) and its derivative, pyridoxal phosphate, a co-factor required for the synthesis of several neurotransmitters – are also discussed.

Three defects of GABA catabolism have been reported: *GABA transaminase deficiency* (which is 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 the γ_2-subunit of the $GABA_A$ receptor are a cause of dominantly inherited epilepsy. Disorders of the metabolism of glycine are discussed in ► Chapter 24.

Seven 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-hydroxylasedeficiency is severe orthostatic hypotension with sympathetic failure. *Dopamine transporter (DAT) defect* causes severe encephalopathy with parkinsonism traits. The other disorders of monoamine metabolism involve both cat-

echolamine and serotonin metabolism. Aromatic *L-amino acid decarboxylase (AADC)* is located upstream of the neurotransmitter amines; treatment of AADC deficiency can be challenging. Mono amineoxidase A (MAO-A) is located downstream; MAO-A deficiency mainly causes behavioural disturbances, and there is no effective treatment for it. Guanosine triphosphate cyclohydrolase-I (GTPCH-I) and sepiapterin reductase (SR) are involved in pterin metabolism upstream of L-dopa and 5-hydroxytryptophan (5-HTP). Both *SR deficiency* and the autosomal dominant form of *GTPCH-1 deficiency* have normal baseline blood phenylalanine concentrations, and effective treatment is available (especially for GTPCH-I deficiency).

Pyridoxine-responsive convulsions, a rare form of early or late infantile seizures are caused by mutations of antiquitin, an enzyme involved in the degradation of lysine (◘ Fig. 23.1). Defective conversion of pyridoxine to pyridoxal phosphate, due to *pyridox(am)ine 5′-phosphate oxidase (PNPO) deficiency*, causes neonatal epilepsy treatable with pyridoxal phosphate.

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 two families [1, 2].

▪ Clinical Presentation

Cardinal clinical features in the Flemish siblings in whom this deficiency was initially observed included intractable seizures, psychomotor retardation, hypotonia with hyperreflexia, lethargy and a high-pitched cry [1]. They showed feeding difficulties from birth, often necessitating gavage feeding. Continued acceleration of lengthwise growth was noted from birth until death, explained by increased fasting plasma growth hormone levels, which could be suppressed by oral glucose. In one patient, head circumference showed a significant increase during the last 6 weeks of life. Postmortem examination of the brain revealed spongiform leukodystrophy. Death ensued at the ages of 25 months and 12 months for the sibs. A Japanese patient similar to those described above was alive at 28 months [2].

▪ Metabolic Derangement

The cerebrospinal fluid (CSF) and plasma concentrations of GABA, GABA conjugates and β-alanine were increased in the Flemish sibship [1]. Liver GABA and β-alanine concentrations were normal. In the Japanese patient the diagnosis was suggested by proton magnetic resonance spectroscopy. Diffusion-weighted MRI revealed hyperintense signals in both internal/external capsules, with extensive subcortical white-matter involvement. The corresponding attenuated diffusion coefficient map confirmed these findings. Deficient GABA-transaminase activity was documented in the liver of one of the Flemish siblings, and also in white cells derived from whole blood of the same patient and the Japanese patient [1, 2]. Intermediate white cell enzyme activities in family members suggested an autosomal recessive inheritance [3]. An isotope-dilution enzyme assay for GABA transaminase suggests that GABA and β-alanine transaminases are identical, explaining the increase of β-alanine in the sibship [4].

▪ Genetics

The gene for GABA transaminase maps to 16p13.3. One patient in the sibship was a compound heterozygote for two missense alleles, c.[659G>A (+) 1433T>C] and p.[Arg220Lys (+) Leu478Pro] [2,5]. In the Japanese case a single missense mutation {c.[275G>A]:p.[Arg92Gln]} was identified [2]. Multiplex probe ligation analysis also revealed an exon deletion in this patient [2].

▪ Diagnostic Tests

The differential diagnosis requires analysis of the relevant amino acids in CSF, primarily free and total GABA, by sensitive techniques [6].Owing to enzymatic homocarnosine degradation, free GABA levels in the CSF show artefactual increases unless samples are rapidly deep-frozen. Enzymatic confirmation can be obtained in lymphocytes, lymphoblasts and liver, and prenatal diagnosis can be achieved in chorionic villus tissue [7].

▪ Treatment and Prognosis

No treatment is available.

29.1.2 Succinic Semialdehyde Dehydrogenase Deficiency

SSADH (aldhehyde dehydrogenase 5a1, ALDH5A1 deficiency, 4-hydroxybutyric aciduria) is the most prevalent of the disorders of GABA metabolism.

▪ Clinical Presentation

The clinical presentation is that of a nonspecific, mild to severe nonprogressive encephalopathy [8-11]. Cardinal manifestations include global developmental delays, with a striking deficit in expressive and developed speech. Ataxia and hypotonia are occasionally encountered, as are seizures (both absence and tonic clonic). Behavioural disturbances may be problematic during adolescence and

adulthood, with features of aggression and obsession-compulsion. Imaging abnormalities may include hyperintensity of the T_1-weighted signals in the globus pallidus, bilaterally, indicative of cytotoxic oedema. Abnormalities of myelination have been noted, and cerebellar atrophy has recently been confirmed in several patients.

▪ Metabolic Derangement

The key feature is an accumulation of γ-hydroxybutyrate (4-hydroxybutyrate) in urine, plasma, and CSF (◘ Fig. 29.1). Additional biochemical abnormalities include increased homocarnosine in the CSF (also documented using magnetic resonance spectroscopy, along with GABA), guanidinobutyrate, D-2-hydroxyglutarate succinic semialdehyde, and 4,5-dihydroxyhexanoic acid (◘ Fig. 29.1). Glutamine levels are low to borderline-low in CSF of patients.

▪ Genetics

The gene for SSADH maps to chromosome 6p22, and the mode of inheritance is autosomal recessive. Multiple disease-associated alleles have been identified, but a mutation hotspot has not been detected. Identification of consanguinity in many families indicates the presence of very rare alleles in human populations.

▪ Diagnostic Tests

Diagnosis is made by determination of γ-hydroxybutyric acid in urine, plasma, and/or CSF. The enzyme deficiency can be demonstrated in white cells employing a fluorometric assay [9]. Prenatal diagnosis may be achieved by combination of methodology (measure of γ-hydroxybutyric acid in amniotic fluid, enzyme assay in amniocytes and/or chorionic villi and molecular genetic analysis).

▪ Treatment and Prognosis

The lifespan of patients does not appear to be shortened, and the oldest known patients are now in their forties. However, some degree of permanent development delay may be expected. All treatments have so far been disappointing, including vigabatrin (Sabril^R; gamma-vinyl-GABA), an irreversible inhibitor of GABA transaminase [12]. Although vigabatrin can reduce γ-hydroxybutyric acid levels, it increases GABA concentrations further and may in addition lead to a permanent reduction in visual fields. $GABA_B$ and GHB receptor antagonists, and also taurine, have shown therapeutic efficacy in a murine model [7,9] but have yet to be employed clinically in patients, and all therapeutic attempts, including those with vigabatrin [12], have been disappointing. The ketogenic diet was very effective at controlling seizures and normalising anthropormorphic abnormalities in the mouse; in patients its use is limited to those with refractory epilepsy.

29.1.3 Homocarnosinosis

Homocarnosinosis is very 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 [13, 14]. In the index family the phenotype was that of a neurological disorder, featuring spastic paraplegia, mental deterioriation and retinal pigmentation. A second case displayed developmental delay, myasthenia, skin desquamation and hypotonia [14]. Skeletal anomalies were associated with a neurological picture that included diffuse brain atrophy and ataxia.

▪ Metabolic Derangement

Concentrations of CSF homocarnosine in the index family (mother and offsrpring) ranged from 50 to 75 μM (normal <3) but were not clearly documented in the second patient.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. The index family suggests an autosomal dominant inheritance, but the exact mode of inheritance remains to be determined.

▪ Diagnostic Tests

First-line investigation includes quantitation of homocarnosine in CSF, although care must be taken to avoid artefactual hydrolysis of homocarnosine. Carnosinase may be readily measured in plasma using either radiometric or amino acid quantitation methodology. (Hydrolysis of carnosine yields L-histidine and β-alanine, which are measurable by amino acid analysis.)

▪ Treatment and Prognosis

Treatment and prognosis remain to be elucidated [13].

29.2 Inborn Defects of Receptors and Transporters of Neurotransmitters

29.2.1 Hyperekplexia

▪ Clinical Presentation

Three main symptoms are required for the diagnosis [15]: (1) a generalised stiffness immediately after birth, which normalises during the first years of life, increases with handling and disappears during sleep; (2) an excessive

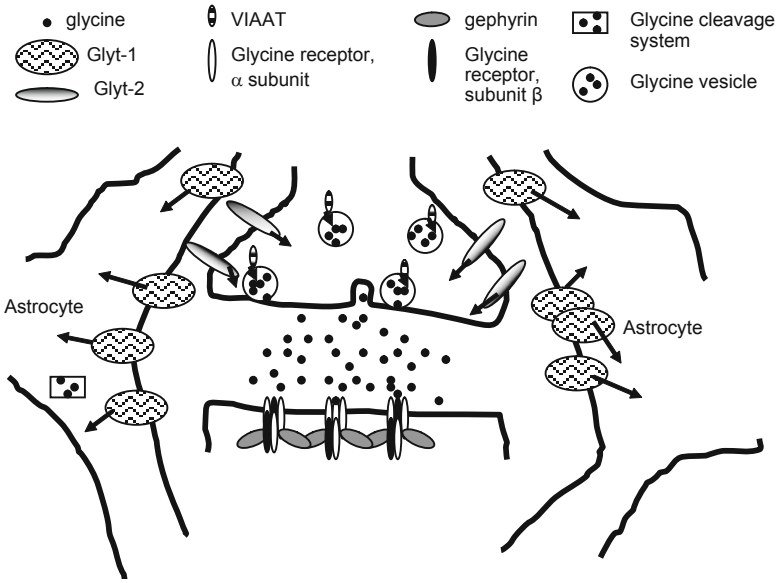


Fig. 29.2. Inhibitory glycinergic synapse. Glycine is stored in vesicles in the presynaptic neuron. Vesicle inhibitory amino acid transporter (*VIAAT*) transports these vesicles to the presynaptic membrane, where glycine is released to the synaptic cleft. Glycine transporters (*GlyT1* and *-2*) are members 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 vesicular inhibitory amino acid transporter (VIAAT)-mediated refilling of re-endocytosed vesicles. The glial isoform GlyT1 removes released glycine from postsynaptic receptirs 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 is the most important cause of hyperekplexia

startle reflex to unexpected stimuli from birth, which in older children causes frequent falls; (3) a short period of generalised stiffness (during which voluntary movements are impossible) following the startle response. Associated features may occur, particularly: (i) an exaggerated head retraction reflex elicited by tapping the tip of the nose; (ii) periodic limb movements during sleep; and (iii) hypnagogic myoclonus (myoclonus occurring when falling asleep). Other symptoms include inguinal, umbilical or epigastric herniations, congenital 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 the signal intensity ratio, *N*-acetylaspartate/creatine + choline, in the frontal cortex, suggesting frontal neuronal dysfunction [16].

Metabolic Derangement

Hyperekplexia is caused by defective inhibitory glycinergic neurotransmission (Fig. 29.2). This may be due to mutations in the genes encoding the α_1-subunit of the glycine receptor (*GLRA1*) [17], the β-subunit of the glycine receptor (*GLRB*) [18], the gene encoding the presynaptic sodium- and chloride- dependent glycine transporter, GlyT2 (*SLC6A5*) [19] and the gene encoding the glycinergic clustering molecule, gephyrin (*GPHN*) [20]. Gephyrin is also involved in molybdenum cofactor (MoCoFa; ►Chapter 36) synthesis [21]. In one individual with hyperekplexia plus severe epilepsy and severe developmental delay, mutations were found in the X-linked *ARHGEF9* gene encoding collybistin, which is also involved in glycinergic receptor clustering [22].

Genetics

Hyperekplexia has, in the great majority of the patients, an autosomal dominant inheritance with near-complete penetrance and variable expression in most pedigrees. With this family history, analysis of the *GLRA1* gene on chromosome 5q32 for point mutations and deletion of exons 1-6 will detect the mutation in approximately 80% of cases [15]. In cases without a family history suggesting dominant inheritance, screening all the genes listed above will detect mutations in approximately 20% of cases; these cases are mostly recessive, with the proband being a compound heterozygote or a homozygote when the parents are consanguineous [15].

Diagnostic Tests

Clinical diagnosis is based on the unique neurological features and the response to medication: the benzodiazepine, 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 the *GLRA1* gene.

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 [23].

29.2.2 GABA Receptor Mutations

- **Clinical Presentation**

Multiple mutations in $GABA_A$ receptor subunits have been reported in three rare causes of idiopathic epilepsy: childhood absence epilepsy (CAE), autosomal dominant epilepsy with febrile seizures plus (ADEFS+), and autosomal dominant juvenile myoclonic epilepsy [24-26].

- **Metabolic Derangement**

Mutations of the GABA receptor alter fast inhibitory neurotransmission facilitated by GABA and altering chloride ion movement. The $GABA_A$ receptor is a ligand-gated membrane pore composed of five distinct GABA subunits (selected from more than 20 cloned receptor subunits) in a variety of configurations in mammalian brain.

- **Genetics**

The mode of inheritance is autosomal dominant, and mutations that map to various chromosomes have been identified in α, β and γ subunits in a number of patients [24-26].

- **Diagnostic Tests**

The diagnosis is based on molecular genetic analysis of different $GABA_A$ receptor subunits.

- **Treatment and Prognosis**

Treatment is generally tailored to the symptoms of the particular epilepsy, but may also address febrile episodes in patients with ADEFS+. The prognosis is also is dependent on the epileptic syndrome involved.

29.2.3 Mitochondrial Glutamate Transporter Defect

This disorder, first described in 2005 [27], is characterised by a severe neonatal onset of 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 temporoparietal regions and abnormal myelination of temporal poles. It is a recessive disorder caused by missense mutations in the *SLC25A22* gene, which encodes a mitochondrial glutamate transporter specifically expressed in the brain during development. The defect impairs oxidation of glutamate. The diagnosis is based on measurement of defective glutamate oxidation in cultured skin fibroblasts and *SLC25A22* mutation analysis [27, 28]. There is no specific treatment.

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 aminoacid-specific hydroxylases, which require tetrahydrobiopterin (BH_4) as a co-factor. BH_4 is also a co-factor of phenylalanine hydroxylase. Its synthesis from GTP is initiated by the rate-limiting GTP cyclohydrolase-1 (GTPCH-I), which forms dihydroneopterin triphosphate (NH_2TP). L-Dopa and 5-hydroxytryptophan (5-HTP) are metabolised by a common B_6-dependent aromatic L-amino acid decarboxylase (AADC) to dopamine (the precursor of the catecholamines, adrenaline and noradrenaline) and serotonin (5-hydroxytryptamine), respectively. Adrenaline and noradrenaline are catabolised 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.

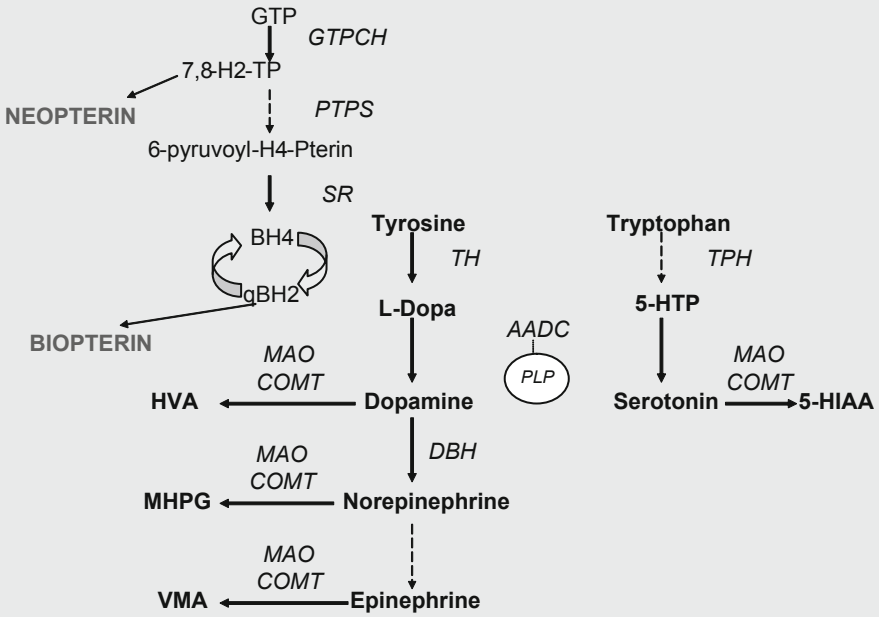


Fig. 29.3. Metabolism of adrenaline, noradrenaline, dopamine, and serotonin: *GTPCH*, GTP cyclohydrolase; *7,8 H2 TP*, dihydroneopterin triphosphate; *PTPS*, 6-pyruvoyltetrahydropterin synthase; *SR*, sepiapterin reductase; *BH4*, tetrahydrobiopterin; *qBH2*, quinonoid dihydrobiopterin; *TH*, tyrosine hydroxylase; *5-HTP*, 5-hydroxytryptophan; *TPH*, tryptophan hydroxylose *AADC*, aromatic L-aminoacid decarboxylase; *PLP*, pyridoxal phosphate (cofactor of AADC); *DBH*, dopamine β-hydroxylase; *MAO*, monoamine oxidase; *COMT*, catechol-*O*-methyltransferase; *HVA*, homovanillic acid; *5-HIAA*, 5-hydroxyindolacetic acid; *MHPG*, 3-methoxy-4-hydroxyphenylglycol; *VMA*, vanillylmandelic acid. Enzyme defects covered in this chapter are depicted by *solid bars*

29.3 Inborn Errors of Monoamine Metabolism

29.3.1 Tyrosine Hydroxylase Deficiency

■ Clinical Presentation

Around 60 patients with tyrosine hydroxylase (TH) deficiency have been reported worldwide [29-35]. Clinically, it causes a neurological disease with predominantly extrapyramidal signs and a variable response to L-dopa. Although different phenotypes have been described (recessive form of Segawa disease, infantile parkinsonism [hypokinetic-rigid syndrome] with dystonia, early-onset progressive encephalopathy), a recent large review has divided them into two main forms: type A, progressive 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 some times epilepsy). Nonprogressive 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 [35]. The motor and cognitive prognosis is worse in type B.

■ Metabolic Derangement

TH converts tyrosine into L-dopa, the direct precursor of catecholamine biosynthesis (◘ Fig. 29.3). This enzymatic step is ratelimiting in the biosynthesis of the catecholamines. The enzyme is expressed in the brain and in the adrenals. 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 level serotonin metabolism is unaffected. HVA levels tend to be lower in more severely affected patients [35, 36].

■ Genetics

TH deficiency is inherited as an autosomal recessive trait. The *TH* gene is located on chromosome 11p15.5. Several mutations including promotor regions [34] and deletions [36] have been described. Common mutations in the Dutch and Greek population have been reported [36, 37].

■ Diagnostic Tests

The most important diagnostic test is the measurement of HVA, MHPG and 5-HIAA in the CSF [38]. As there is a lumbosacral gradient in the concentration of HVA and 5-HIAA, measurements should be carried out in a standardised CSF volume fraction in an experienced laboratory. 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. Elevated prolactin in blood is not constant. Molecular analysis of the *TH* gene is available. Structural variant analysis may be helpful [36].

■ Treatment and Prognosis

In most cases, TH deficiency can be treated with low-dose L-dopa in combination with an L-dopa decarboxylase inhibitor. However, the response is variable, ranging from complete remission (more likely in type A phenotype) to mild improvement. Therapy should be started with low doses of L-dopa, initially 1-2 mg/kg per day in four to six divided doses, and only increased over periods of weeks (at least 2 weeks) or months, since these patients are especially prone to major side effects even on low doses (mainly irritability, dyskinesia and ballismus). It may take weeks to months of careful treatment with increasing doses before a positive effect can be convincingly demonstrated. It is not always possible to normalise the catecholamine levels in the CSF.

29.3.2 Aromatic L-Aminoacid Decarboxylase Deficiency

■ Clinical Presentation

Since its first description in 1988, aromatic L-aminoacid decarboxylase(AADC) deficiency has been reported in almost 80 patients worldwide [39-42]. Neonatal symptoms are reported in over half (poor sucking and feeding difficulties, lethargy, increased startle response, hypothermia, ptosis), but all patients develop neurological signs within the first 6 months of life. Patients may present with a severe and progressive epileptic encephalopathy, although epilepsy is rarely a single symptom [43, 44]. The most frequent signs are truncal hypotonia associated with limb rigidity, oculoryric crises and developmental delay [42]. Dystonia, ptosis and autonomic dysfunction (temperature instability with hypothermia, gastrointestinal symptoms, paroxysmal sweating and impaired heart rate and blood pressure regulation) will also develop. Initial suspected diagnoses included cerebral palsy, epilepsy, hyperekplexia and a mitochondrial disorder.

■ Metabolic Derangement

AADC is implicated in two metabolic pathways: the biosynthesis of catecholamines and that of serotonin (◘ Fig. 29.3). The activity of the homodimeric enzyme requires pyridoxal phosphate as a co-factor. 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 and 5-HTP, and products derived from them, 3-methoxytyrosine and vanillyllactic acid (VLA). Often the finding of increased VLA in the urinary organic acid profile is the first important clue to this diagnosis. In several patients a paradoxical hyperdopaminuria has been noted, which is probably due to production of dopamine and metabolites in nonneural cells.

▪ Genetics

AADC deficiency is inherited as an autosomal recessive trait. The *AADC* gene has been assigned to chromosome 7p11. Mutations in the gene have been reported, with IVS6+ 4A>T being the most common [42].

▪ Diagnostic Tests

L-Dopa, 3-methoxytyrosine, VLA and 5-HTP can be found elevated in urine, CSF and plasma. The increase of VLA may be observed in the urinary organic acid profile. Also, low concentrations of HVA, 5-HIAA and MHPG in CSF may lead to the diagnosis. AADC deficiency can easily be confirmed by finding a deficiency of the enzyme in plasma. Although this pattern strongly resembles that found in pyridoxine phosphate oxidase (PNPO) deficiency, AADC deficiency does not cause the other metabolic abnormalities seen in that condition (► Section 29.4.2).

▪ Treatment and Prognosis

Treatment in AADC deficiency may be beneficial, but the effects are limited and the long-term prognosis is poor. Some patients with relatively mild forms clearly improved on combined therapy with pyridoxine (B_6)/pyridoxal phosphate, dopamine agonists and monoamine oxidase B inhibitors [42].

29.3.3 Dopamine β-Hydroxylase Deficiency

▪ Clinical Presentation

This defect is characterised by severe orthostatic hypotension; supine blood pressure is normal to low. Hypotension may lead to recurrent episodes of fainting, and most patients complain of fatigue and impaired exercise tolerance. Although dopamine β-hydroxylase (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 [45]. Differential diagnosis includes pure autonomic failure/autonomic neuropathy, familial dysautonomia, and Shy-Drager syndrome or central autonomic failure. DBH is a copper-dependent enzyme, and thus DBH activity is depressed in ATP7A-related copper transport disorders (Menkes disease and occipital horn syndrome; ► Chapter 38).

▪ Metabolic Derangement

DBH converts dopamine to noradrenaline. It is present in the synapses of postganglionic sympathetic neurons. A defect in the enzyme should have consequences for (nor-) adrenergic neurons and also for the adrenals.

▪ Genetics

The *DBH* gene is located on chromosome 9q34. Pathogenic mutations have been found in all known patients with symptomatic DBH deficiency.

▪ Diagnostic Tests

Tests of autonomic function may provide diagnostic information of very high specificity [46, 47].The patients typically have extremely low plasma noradrenaline and adrenaline levels and increased or high-normal levels of dopamine. 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) [47].

▪ Treatment and Prognosis

Therapy with L-dihydroxyphenylserine (L-dops) is available. This compound can be directly converted by AADC into noradrenaline, thereby bypassing the defective enzyme. Administration of 100-500 mg L-dops orally twice or three times daily increases blood pressure and restores plasma NE levels; however plasma epinephrine concentration still remains below a detectable level [45]. The prognosis on therapy is satisfactory to good.

29.3.4 Monoamine Oxidase-A Deficiency

▪ Clinical Presentation

Monoamine oxidase-A (MAO-A) deficiency or Brunner syndrome has been identified in five generations of one Dutch family [48, 49]. Only males were affected. They

showed borderline mental retardation with prominent behavioural disturbances, including aggressive and sometimes violent behaviour, arson, attempted rape and exhibitionism. The patients were nondysmorphic and had a tendency to stereotyped hand movements. Additionally, a functional polymorphism of the *MAO-A* gene promoter region may act as a genetic modifier of the severity of autism in males [50]. Furthermore, other MAO-A polymorphisms have been related to abnormal limbic circuitry for emotion regulation and cognitive control, explaining impulsive aggression and serious delinquency [51-53].

MAO exists as two isoenzymes (A and B). The genes encoding for both isoenzymes are located on the X-chromosome. 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 [54]. They are severely mentally retarded and blind. Patients with only the *MAO-B* and the Norrie genes affected are not mentally retarded and do not have abnormalities of catecholamine metabolites in urine. Elevated excretion of phenylethylamine as a specific MAO-B substrate is a consistent finding in patients affected with the contiguous-gene syndrome.

▪ Metabolic Derangement

MAO-A is the isoenzyme found in neural tissue. Its deficiency causes a defect in the catabolism of both serotonin and the catecholamines.

▪ Genetics

The locus for this X-linked inherited disease has been assigned to Xp11.21. A point mutation in the eighth exon of the *MAO-A* gene, causing premature truncation of the protein, has been found in this family [48, 49].

▪ Diagnostic Tests

Elevation of urinary serotonin, normetanephrine, metanephrine and 3-methoxytyramine is the characteristically abnormal excretion pattern in random urine samples of the patients. The ratios in urine of normetanephrine to VMA, or normetanephrine to MHPG, are abnormally high [55]. The HVA/VMA ratio in urine (patients >4) may also provide a first indication for this diagnosis. 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.

▪ 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

Guanosine triphosphate cyclohydrolase-I (GTPCH-I) and sepiapterin reductase (SR) deficiencies are disorders of pterins. In SR deficiency and the autosomal dominant form of GTPCH-1 deficiency the baseline phenylalanine (Phe) is normal. In some peripheral tissues, other enzymes, such as aldose reductase, carbonyl reductase and dihydrofolate reductase, produce sufficient BH^4 to maintain the Phe hydroxylase activity, thereby avoiding hyperphenylalaninaemia. However, in brain these enzyme activities are very low, insufficient for the synthesis of normal amounts of BH_4. Four other pterin deficiencies are associated with hyperphenylalaninaemia and can be detected by newborn screening programmes (► Chapter 17).

▪ Clinical Presentation

In 1994, autosomal dominant GTPCH-I deficiency was identified as the cause of dopa-responsive dystonia (DRD) [56]. Initially, it was published under the name »hereditary progressive dystonia with marked diurnal fluctuation« [57], and prior to the identification of the causative gene this disease was called Segawa syndrome [58]. Patients with this deficiency develop symptoms during the 1st decade of life. The onset age in childhood is around 6 years. However, there are patients who have an onset as early as the 1st week of life or in adulthood, even at ages older than 50 years [59]. Dystonia in the lower limbs is the initial and most prominent symptom. Unless treated with L-dopa, the dystonia becomes generalised. Diurnal fluctuation of the symptoms with improvement after sleep is a feature in most patients. In general, the disease is classified into two types, the postural dystonia type and the action dystonia type, which is characterised by vigorous dystonic movements [59]. Dystonia might also have a relapsing and remitting course, and may be associated with oculogyric crises, depression and migraine. Paroxysmal exercise-induced dystonia has been described in one family [60]. As a rule, symptomatology is asymmetrical. Adult-onset patients can start with hand tremor and gait disturbance due to generalised rigidity. Recessive 'DRD' may be caused by TH deficiency (it was recently classified as DYT5), SR deficiency and the recessive form of GTPCH-I deficiency. Recessive GTPCH-I deficiency is usually associated with hyperphenylaninaemia and detected by newborn screening (► Chapter 17), but a few cases have been published in which the patients presented with DRD [61-64]. The clinical spectrum ranges from the classic DRD to severe neonatal forms similar to TH deficiency type B.

■ **Metabolic Derangement**

GTPCH-I is the initial and rate-limiting step in the biosynthesis of BH_4, the essential co-factor of various aromatic amino acid hydroxylases (◘ Fig. 29.3) with the highest affinity for TH. Therefore, the deficiency state of the enzyme is characterised by defective biosynthesis of serotonin and catecholamines. For the other defects of biopterin metabolism, the reader is referred to ►Chapter 17.

■ **Genetics**

The gene for GTPCH-I is located on chromosome 14q22.1-q22.2. GTPCH-I deficiency can be inherited as an autosomal dominant trait with 30% penetrance. The female-to-male ratio is approximately 3:1. More than 100 mutations have been found as causes for the dominant form of the disease.

■ **Diagnostic Tests**

Some patients with the recessive form of the disease may be diagnosed through hyperphenylalaninaemia found on neonatal screening. Patients with dominant GTPCH-1 deficiency escape detection during the newborn screening, as they 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 to about 20-30% of normal levels). (2) Measurement of CSF HVA and 5-HIAA. A normal or slightly low CSF HVA in combination with a low or slightly low 5-HIAA may be meaningful. (3) An oral Phe loading test. In general, it reveals a 6-h increase in Phe levels. Phe/Tyr ratios remain elevated during the post-loading period, while biopterin levels decline. (4) Mutation analysis. (5) Measurement of the enzyme activity in fibroblasts.

■ **Treatment and Prognosis**

Patients have been treated successfully with a combination of low-dose L-dopa (4-5 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, levodopa does not always produce a complete response [59].

29.3.6 Sepiapterine Reductase Deficiency

SR deficiency is an autosomal recessive disease implicated in the final step of BH_4 synthesis. Fewer than 25 cases have been described [65]. Usually, the onset of the disease occurs in the 1st decade of life and the clinical picture includes severe encephalopathy with parkinsonism-dystonia traits, seizures, temperature instability, sleep disturbances and DRD phenotype. CSF study shows high levels of biopterin and sepiapterin with normal levels of neopterin and low concentrations of HVA and 5-HIAA (that can be found also in urine). Enzyme activity in fibroblasts is reduced, and patients can develop hyperphenylalaninaemia on oral challenge with Phe. Diverse mutations have been described. Treatment with L-dopa and 5-hydroxytryptophan notably improves the clinical picture.

29.3.7 Dopamine Transporter Defect

This new defect has been described in 11 children presenting in infancy with either hyperkinesia, parkinsonism or a mixed hyperkinetic/hypokinetic movement disorder. All of them developed severe parkinsonism-dystonia, abnormal eye movement disorders and pyramidal tract features, and all had a raised ratio of homovanillic acid to 5-hydroxyindoleacetic acid [66]. Excretion of urinary HVA acid was slightly increased. CT DaTSCAN showed loss of dopamine transporter activity in the basal nuclei. All of them showed a poor clinical response to multiple therapeutic agents. *SLC6A3* mutations were identified, and in all missense mutations loss of function was recorded in in vitro functional studies. Thus, in humans, *SLC6A3* mutations that impair DAT-mediated dopamine transport activity are associated with an early-onset non-dopa-responsive complex movement disorder [66].

29.3.8 Other Inborn Defects Involved in Monoamine Metabolism

Although the pathophysiology is not always clear, several IEM have been associated with secondary monoamine deficiencies. Low CSF HVA levels have been found in Lesch-Nyhan disease [67] and mitochondrial disorders [68], whereas low 5-HIAA levels have been found in Niemann-Pick type C, X-linked adrenoleukodystrophy, some organic acidurias and mitochondrial disorders [69]. Different genes (*SNCA*, *PARK2*, *PINK1*, *PARK7* and *LRRK2*) and autosomal dominant striatal degeneration due to mutations in the phosphodiesterase 8B gene have been reported as responsible for juvenile- and adult-onset parkinsonism [70,71], whereas FBXO7 mutations cause autosomal recessive early-onset parkinsonian-pyramidal syndrome [72].

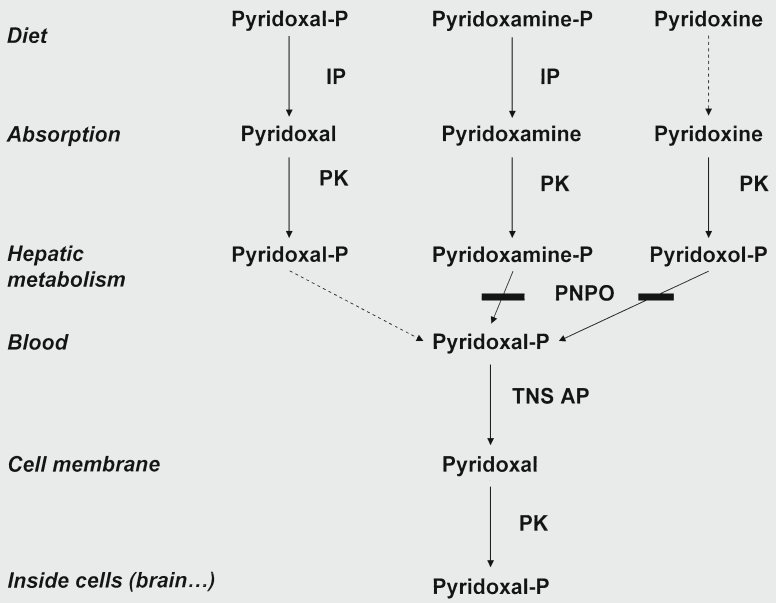
◘ Table 29.1 shows the main clinical and biochemical phenotypes in disorders of biogenic amines.

Table 29.1. Clinical and biochemical phenotypes in biogenic amine disorders

Clinical phenotypes	Test in	Basic biochemical profiles	Diagnosis
Hypokinetic-rigid syndrome (+/- other neurological signs) Dopa-responsive-dystonia (postural or action dystonia) Complex encephalopathies of unknown origin, especially if some of the following signs are present: oculogyric crisis, abnormal movements, ptosis, autonomic dysfunction, seizures or seizure-like episodes	**CSF**	Low HVA	– TH deficiency – Consider different genes related to early (*FBXO7*) and juvenile/adult onset parkinsonism (*SNCA*, *PARK2*, *PINK1*, *PARK7*, *LRRK2*, phosphodiesterase 8B) – Consider secondary causes: other IEM (Lesch-Nyhan, mitochondrial disorders, neurodegenerative disorders), different acquired conditions such as hypoxic encephalopathy – If low neopterin and biopterin are associated: GTPCHI deficiency
		High HVA	– DAT deficiency – Consider secondary causes: SSADH deficiency, Kearns-Sayre, hydrocephalus – DBH and MAO A deficiencies have been reported to have high CSF HVA, although clinical phenotypes are very different – If high VLA, L-dopa, 3-methoxytyroxine and 5-HTTP: AADC deficiency
		Low HVA and 5HIAA	– Consider PNPO deficiency although it causes neonatal epileptic encephalopathy – If high levels of biopterin and sepiapterin with normal levels of neopterin: SR deficiency – If low levels of neopterin and biopterin: GTPCH-1 deficiency (HVA and 5HIAA could be slightly low. 5HIAA may be normal)
		Low neopterin and biopterin	– Without alterations of other metabolites: consider secondary causes such as mitochondrial disorders, leukodystrophies, epileptic encephalopathies, hypoxia – GTPCHI deficiency: HVA is low; 5HIAA could be low/slightly low/normal
Severe orthostatic hypotension Perinatal hypoglycaemia, hypotension and hypothermia	**Urine**	– High ratios normetanephrine/VMA, normetanephrine/MHPG – Ratio HVA/VMA >4	– DBH deficiency
Borderline mental retardation with behavioural abnormalities (aggressive, violent behaviour)	**Urine**	– High serotonin, normetanephrine, 3-methoxytyramine and tyramine	– MAO-A deficiency

Pyridoxine and Pyridoxal Phosphate

Pyridoxal 5′-phosphate (pyridoxal-P), the coenzyme form of vitamin B_6, is the co-factor for numerous enzymes involved in neurotransmitter metabolism. A reduced concentration of pyridoxal-P in the brain can lead to epilepsy and to the following abnormalities in the CSF: low HVA and 5HIAA with increased 3-*O*-methyl-DOPA, raised glycine, threonine. Pyridoxal-P can be formed from pyridoxine or pyridoxamine by the action of two enzymes, a kinase: pyridoxal kinase (PK) and an oxidase: pyridox(am)ine 5′-phosphate oxidase (PNPO) (◘ Fig. 29.4). Formation of pyridoxal-P from dietary pyridoxal or dietary pyridoxal-P requires only pyridoxal kinase.



◘ **Fig. 29.4.** Conversion of dietary vitamin B_6 to intracellular pyridoxal 5′-phosphate cofactor: *IP*, intestinal phosphatases; *P*, 5′-phosphate; *PK*, pyridoxal kinase; *PNPO*, pyridox(am)ine phosphate oxidase; *TNS AP*, tissue nonspecific alkaline phosphatase. The enzyme block is indicated by a *solid bar*

29.4 Inborn Disorders Involving Pyridoxine and Pyridoxal Phosphate

Pyridoxine-responsive epilepsy (PRE) has been attributed to reduced activity of glutamate decarboxylase, but this hypothesis has never been proven. In 2006, it was found to be caused by mutations of antiquitin, an enzyme involved in the degradation of lysine (◘ Fig. 23.1). In 2005 a new disorder was identified, with neonatal seizures resistant to anticonvulsants and pyridoxine but responsive to pyridoxal phosphate. The defect was found to be in PNPO.

29.4.1 Pyridoxine-responsive Epilepsy

Pyridoxine-responsive epilepsy was first reported in 1954 [73]. It has been considered a rare cause of convulsions in early childhood, fewer than 100 probands having been reported up to 2001 [74]. However, recently, some quite large series have been reported [75, 76]

▪ Clinical Presentation

The clinical diagnosis of classic pyridoxine-responsive epilepsy has been based on the following criteria:

- Onset of convulsions before or within a month of birth.
- Refractory to anticonvulsant drugs.
- Rapid response to pyridoxine (50-100 mg).
- Seizure control requires a maintenance dose (5-10 mg/kg/day). Seizures return within a few days of stopping this maintenance dose.

Atypical presentations of PRE [74] differ as follows:

- Later onset of the attacks (up to the age of about 2 years).

- Up to 7 days of treatment may be required before seizures respond.
- Prolonged seizure-free intervals without pyridoxine (as long as 5 years).
- Need for larger pyridoxine doses in some patients.

In a series of 37 patients with confirmed antiquitin mutations [76], the following clinical features were observed: parental consanguinity in 25%; abnormal fetal movements (intrauterine seizures) in 33%; fetal distress prior to delivery in 29%; Apgar score <7 at 1 min in 15%; neonatal acidosis in 26%; respiratory distress in 33%; hypotonia in 57%; abdominal distension/vomiting in 27%; irritability in 58%; onset of seizures within first 28 days in 89%; resistance to antiepileptic drugs – complete in 58%, partial in 38%; clonic seizures in 91%; tonic seizures in 44%; myoclonic jerks in 62%; cardiovascular / respiratory decompensation with first administration of pyridoxine in 27%; complete cessation of seizures with first trial of pyridoxine in 86%. Patients with seizures well controlled by pyridoxine treatment showed speech delay, (58%); strabismus (33%); motor delay (75%); and breakthrough seizures with fever (35%). The time interval between withdrawal of pyridoxine and recurrence of seizures ranged from 1 to 51 days. Additional features, observed infrequently in early infancy, included thrombosis, *E. coli* sepsis, hypocalcaemia with or without hypomagnesaemia, diabetes insipidus, optic nerve hypoplasia, hypothyroidism and hydrocephalus. EEG findings were very variable, but (before treatment) included burst suppression and hypsarrhythmia. MRI findings included normal appearance; reduced white matter bulk, e.g. of corpus callosum and cerebellar white matter; cortical dysplasia; changes indistinguishable from those seen in periventricular leukomalacia; and ventriculomegaly (hydrocephalus).

Metabolic Derangement

Pyridoxine-responsive convulsions were first attributed to brain GABA deficiency.

In 2000, Plecko et al. detected elevated CSF pipecolic acid in pyridoxine-dependent epilepsy [77]. This led Mills et al. [78] to speculate that PRE is caused by a defect in the pathway of lysine catabolism via pipecolic in the brain (Fig. 23.1). They predicted accumulation of Δ^1-piperideine 6-carboxylic acid (P6C; the compound immediately after pipecolic acid in this pathway) and inactivation of pyridoxal phosphate by P6C. Comparative genomics identified a human gene, *ALDH7A1*, encoding antiquitin, which was shown to act as a dehydrogenase active on P6C and its equilibrium partner (α-aminoadipic semialdehyde, α-AASA). The *ALDH7A1* gene was located on chromosome 5q31 – previously shown by linkage to be the major locus for PRE [61]. Children with PRE had mutations in the *ALDH7A1* gene which, when expressed in CHO cells, produced activity of α-AASA/P6C dehydrogenase that was <2% of that produced by wildtype DNA. Children with PRE had increased concentrations of α-AASA in CSF, plasma and urine. A synthetic equilibrium mixture of P6C and α-AASA, or the urine of patients with PRE, could inactivate pyridoxal phosphate by a condensation reaction. Thus PRE is explained by a defect in the lysine catabolism pathway in the brain that leads to accelerated loss of pyridoxal phosphate. Affected individuals need an increased intake of pyridoxine to compensate for the inactivation of pyridoxal phosphate in the brain.

Genetics

In its typical form the disease has an autosomal recessive inheritance, and there is evidence that this also holds true for the later onset presentation. Both the linkage studies [79] and the *ALDH7A1* mutation studies [76, 78] indicate that mutations in the *ALDH7A1* gene on 5q31 are the major cause of PRE. The mutations so far described include missense, nonsense and splice site mutations, a single base-pair insertion and deletions of 1-4 base pairs; affected individuals were either homozygous for a mutation or compound heterozygotes for two mutations. Approximately 60% of mutations are in the exons 4,6,9,11 and 14 (or their exon/intron boundaries). The commonest mutation (responsible for ca. 30% of mutated alleles) is E399Q (exon 14).

Diagnostic Tests

PRE due to deficiency of antiquitin can be reliably diagnosed by measurement of urinary α-AASA followed by *ALDH7A1* mutation screening [76]. α-AASA and pipecolic acid are also elevated in plasma and CSF, but elevation of pipecolic acid is not specific to PRE. However, neonatal epileptic encephalopathy is a medical emergency and decisions usually need to be made about ongoing treatment before the results of specialised laboratory tests are available. Therefore, the clinical and electrophysiological response to pyridoxine treatment remains an important diagnostic test. A clear-cut response to pyridoxine is most likely to be seen in a neonate in status epilepticus with clear abnormalities in the EEG (e.g. burst-suppression). However, a trial of pyridoxine should ideally be performed in all seizure disorders with onset before the age of 18 months and where the aetiology is unclear. A trial of pyridoxine in the neonate should always be conducted in an intensive care unit; cessation of seizures may be accompanied, for the first few hours, by neurological depression with an isoelectric EEG, and

respiratory and/or cardiovascular depression can also occur. Until recently, confirmation of the diagnosis of PRE required temporary cessation of treatment to show that seizures recurred [74]. For the majority of children, this provocation testing can be replaced by measurements of α-AASA and sequencing of *ALDH7A1*.

- **Treatment and Prognosis**

With the infant in an intensive care setting and with full EEG monitoring, 50-100 mg of pyridoxine is given i.v. If the infant has PRE, cessation of seizures will normally occur within minutes. Apnoea, profound hypotonia and hypotension are most likely to occur in an infant who has already been loaded with antiepileptic drugs such as phenobarbitone [74]. If the pyridoxine is given enterally, fits usually stop within hours. After a single dose of 100 mg of pyridoxine, an infant will usually remain fit free for 2-5 days. Permanent control of seizures usually requires oral pyridoxine at a daily dose of 5-10 mg/kg. Untreated, PRE will usually lead to continuing anticonvulsant-resistant seizures and can be fatal (at least in the neonatal-onset form). On daily pyridoxine treatment most children remain seizure free (with occasional breakthrough seizures precipitated by febrile illness). They often show mild to moderate learning difficulties, however, with speech particularly affected. In one child with learning difficulties there was a significant improvement in IQ when the daily dose of pyridoxine was increased from 5 to 15 mg/kg [74, 76]. Very high doses of pyridoxine can produce peripheral neuropathy, so nerve conduction times should be monitored.

Individuals with PRE can have seizures in utero. Attempts have been made to prevent this by treating pregnant women who already have a child affected by PRE. The daily dose of pyridoxine has varied from 9 mg to 110 mg; some authors suggest that this in utero treatment helps prevent subsequent developmental delay in the child [74].

29.4.2 Pyridox(am)ine 5′-Phosphate Oxidase Deficiency

- **Clinical Presentation**

PNPO deficiency causes severe neonatal epileptic encephalopathy. Sixteen affected infants from eight families have been reported [80-84]. In five of the eight families, the parents were consanguineous; male and female infants were equally affected. Clinical features were: fetal seizures, 3/16; fetal distress prior to delivery, 5/16; premature birth, mean 32 weeks of gestation; low Apgar score/requirement for intubation at delivery, 5/16; onset of seizures – in first 24h, 11/14; between 24h and 72h, 2/14; between 72h and 2 weeks 1/14; burst-suppression pattern on EEG, 10/11; seizures completely resistant to antiepileptic drugs, 13/16; complete resistance to pyridoxine, 7/10; acidotic 6/16; raised blood lactate 8/16; distressing spasms (?dystonia), 3/16; anaemia, 3/16; hepatomegaly, 3/16; abdominal distension, 2/16; hypoglycaemia 2/16.

Much larger numbers of infants have been described in whom severe epilepsy has been better controlled with the use of pyridoxal-P than with the use of pyridoxine. It is not yet known whether any of these infants have mutations or polymorphisms in the *PNPO* gene [81].

- **Metabolic Derangement**

In patients who lack PNPO, the active form of vitamin B_6 (pyridoxal-P) cannot be synthesised from dietary pyridoxine (present in vegetables and added as the B_6 supplement to infant formulae and parenteral nutrition). Nor can it be regenerated by recycling of pyridoxamine phosphate. A low concentration of pyridoxal-P in the brain leads to reduced activity of a large number of pyridoxal-P-dependent reactions, and this can lead to a full spectrum of abnormalities of amino acids and amine neurotransmitter metabolites in the CSF. The affected enzymes include aromatic aminoacid decarboxylase, the glycine cleavage enzyme, threonine dehydratase and histidine decarboxylase.

- **Genetics**

The *PNPO* gene is situated on chromosome 17q21.2. The first patients described were shown to be homozygous for missense, splice site and stop codon mutations [80]. Expression studies showed that the splice site (IVS3-1-g>a) and stop codon (X262Q) mutations were null activity mutations and that the missense mutation (R229W) markedly reduced PNPO activity. Subsequently described mutations include: a homozygous missense mutation, p.R95H [82,83]; a very similar homozygous missense mutation, p.R95C; compound heterozygosity for a missense mutation, p.D33V and a single base pair deletion, c.246delT [82]; and a homozygous nonsense mutation, p.A174X [84].

- **Diagnostic Tests**

The least invasive test for PNPO deficiency is measurement of urinary VLA, but the sensitivity and specificity of this determination in neonates with seizures has yet to be determined. Other results pointing to low pyridoxal phosphate levels in cells, particularly in the brain, include low CSF HVA and 5HIAA with high CSF 3-methoxytyrosine, glycine, threonine, histidine and taurine, and low plasma arginine. CSF pyridoxal phosphate and pyridoxal

concentrations are low in PNPO deficiency. However, a very similar picture can be seen when low brain pyridoxal-P levels are caused by other inborn errors, such as antiquitin deficiency (see above), and could even be caused by severe dietary B_6 deficiency. Improved methods for measurement of pyridoxine, pyridoxamine and pyridoxal and their phosphates are likely to prove important in determining the cause of pyridoxal-P deficiency in the future. For the moment a therapeutic trial of pyridoxal-P remains an important means of diagnosis. The best way of confirming the diagnosis is by sequencing the *PNPO* gene. This should certainly be done if a neonate has seizures that respond dramatically to pyridoxal-P after failing to respond to pyridoxine.

- **Treatment and Prognosis**

Untreated, 11 out of 12 patients with PNPO deficiency have died at under 1 year of age; 1 girl survived to 3 years, with severe epilepsy and developmental delay. Pyridoxal-P has been very effective in controlling seizures in PNPO deficiency, and some patients are showing normal development; however, patients who had uncontrolled seizures for several days have gone on to show moderate to severe neurological handicap, and one died in early infancy, having already been very ill when treatment was started. Pyridoxal-P for intravenous use is not readily available. Fortunately, pyridoxal-P is very effective when given via a nasogastric tube (in a sick neonate) or orally following recovery from the seizures. A trial of treatment with pyridoxal-P should only be undertaken in a setting where full resuscitation and intensive care facilities are available. A 50-mg dose will usually lead to cessation of seizures within the hour, but may also produce hypotension and profound hypotonia and unresponsiveness, which may take a few days to resolve [80]. Continuing control of seizures has usually been achieved with 10 mg/kg of pyridoxal-P 6-hourly, but higher and more frequent doses have been required to control seizures fully in some patients. One patient has developed signs of liver damage while on high-dose pyridoxal-P treatment. It is recommended that each dose is freshly made up by dissolving the tablets in water immediately before administration.

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Disorders in the Metabolism of Glutathione and Imidazole Dipeptides

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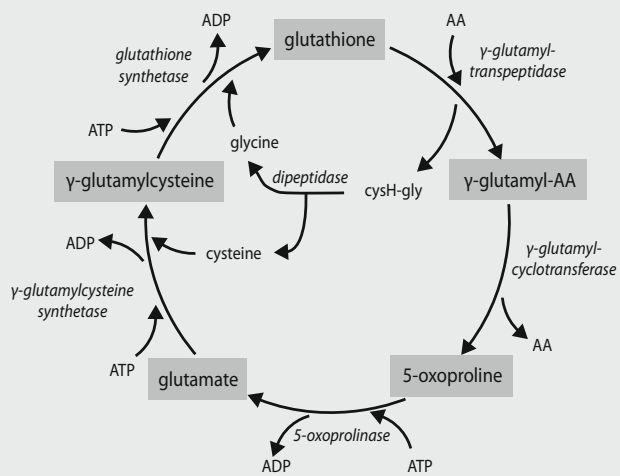
Glutathione and Imidazole Dipeptide Metabolism

Glutathione 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 and amino acid transport. Glutathione is synthesised and metabolised in the γ-glutamyl cycle, in which six enzymes take part in its synthesis and turnover. It is synthesised from glutamate by sequential actions of γ-glutamylcysteine synthetase and glutathione synthetase. Degradation of glutathione 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 glutathione is feedback regulated, i.e. glutathione acts as an inhibitor of γ-glutamylcysteine synthetase.

Imidazole dipeptides derive their name from the presence of the imidazole ring of histidine. *Carnosine* (β-alanyl-histidine) is found in skeletal (but not cardiac) muscle and brain, where it may be a neurotransmitter. It is hydrolysed by two isozymes. Cytosolic carnosinase is present in most human tissues and displays a very broad dipeptidase specificity but does not hydrolyse anserine or homocarnosine. Serum carnosinase (also found in cerebrospinal fluid) hydrolyses carnosine and anserine but hydrolyses homocarnosine very poorly. Its activity increases gradually with age.

Anserine (β-alanyl-1-methylhistidine) is normally absent from human tissues and body fluids, but may be derived from the diet (particularly in infants, owing to their low serum carnosinase activity), or may be found in patients with serum carnosinase deficiency. Anserine is found is skeletal muscles of birds and certain mammals, such as rabbits. Its physiological function is unclear.

Homocarnosine (γ-aminobutyryl-histidine) is a brain-specific dipeptide. It is hydrolysed by serum but not by cytosolic carnosinase. Cerebrospinal fluid homocarnosine concentrations are higher in children (approx. 8 μM) than in adults (approx. 1 μM). The physiological function of homocarnosine is unknown. It may act as a reservoir for γ-aminobutyric acid (GABA) in some parts of the brain (◘ Fig. 30.1).



◘ **Fig. 30.1.** The γ-glutamyl cycle. *AA*, amino acid

Genetic defects in humans have been described in five of the six enzymes of the γ-glutamyl cycle. *Glutathione synthetase deficiency* is the most common recognised disorder. The most severe form is associated with metabolic acidosis, haemolytic anaemia, 5-oxoprolinuria and central nervous system (CNS) damage. *γ-Glutamylcysteine synthetase deficiency* is also associated with haemolytic anaemia, and in some cases with disturbed neuromuscular function and generalised aminoaciduria. *γ-Glutamyl transpeptidase deficiency* has been found in some patients with CNS involvement and glutathionuria. *5-Oxoprolinase deficiency* is associated with 5-oxoprolinuria, without, however, a clear association with other clinical

symptoms. *Dipeptidase deficiency* has been suggested in only one patient worldwide, with mental retardation, mild motor impairment, and partial deafness.

Serum carnosinase deficiency and *homocarnosinosis* are probably the same disorder. It is uncertain whether there is a relationship between the biochemical abnormalities and clinical symptoms. *Prolidase deficiency* causes skin lesions and recalcitrant ulceration (particularly on the lower legs) in addition to other features, such as impaired psychomotor development and recurrent infections. The severity of clinical expression is highly variable.

30.1 Disorders in the Metabolism of Glutathione

30.1.1 γ-Glutamylcysteine Synthetase Deficiency

▪ Clinical Presentation

Up to now, 9 patients in seven different families have been identified worldwide. The disease is characterised in all patients by haemolytic anaemia, usually rather mild. In two patients spinocerebellar degeneration, peripheral neuropathy and myopathy have been reported as additional symptoms [1]. Further symptoms reported in association with this metabolic defect have included transient jaundice, reticulocytosis, hepatosplenomegaly and delayed psychomotor development.

▪ Metabolic Derangement

γ-Glutamylcysteine synthetase catalyses the first and rate-limiting step in the synthesis of glutathione (GSH, ◘ Fig. 30.1). Its deficiency results in low levels of cellular GSH and γ-glutamylcysteine content. Knock-down of γ-glutamylcysteine synthetase in rat causes acetaminophen-induced hepatotoxicity.

▪ Genetics

This disease is transmitted as an autosomal recessive trait. γ-Glutamylcysteine synthetase is a dimer consisting of two nonidentical subunits encoded by two separate genes, which are located on chromosomes 1p21 (light or regulatory subunit) and 6p12 (heavy or catalytic subunit), respectively. The heavy subunit with a molecular weight about 73kDa exhibits the catalytic activity of the native enzyme and is also responsible for the feedback inhibition by GSH. The light subunit with a molecular weight about 28 kDa is catalytically inactive; however, it has an important regulatory role. Up to now, four different mutations all in the heavy subunit have been identified in four families with this enzyme deficiency.

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. 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 in erythrocytes, leukocytes and/or cultured skin fibroblasts. In addition, low levels of GSH and γ-glutamylcysteine are found in erythrocytes and/or cultured skin fibroblasts. Mutation analysis of the γ-glutamylcysteine synthetase genes confirms the diagnosis. Patients with mutational analysis published so far all had homozygous mutations in the gene encoding the heavy subunit of the enzyme. In erythrocytes heterozygous carriers showed a γ-glutamylcysteine synthetase activity about 50% of normal levels, whereas intracellular GSH content was normal.

▪ Treatment and Prognosis

Affected patients should avoid food and drugs known to precipitate haemolytic crises in patients with glucose-6-phosphate dehydrogenase deficiency, e.g. fava beans, sulfonamides, acetylsalicylic acid, phenobarbital. No further studies have been made. The prognosis remains to be established.

30.1.2 Glutathione Synthetase Deficiency

▪ Clinical Presentation

Deficiency of glutathione synthetase (GS) is the most common and best-characterised inborn error of GSH metabolism. More than 70 patients from 55 families have been reported worldwide. According to the severity of clinical symptoms, GS deficiency are classified as mild, moderate or severe [2]. Patients with mild GS deficiency show mild haemolytic anaaemia 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 haemolytic anaemia, jaundice and 5-oxoprolinuria. After the neonatal period, the condition usually stabilises, but patients may become critically ill during infections owing to pronounced acidosis and electrolyte imbalances. Several patients have died during such episodes. In addition to the symptoms mentioned above, patients with severe GS deficiency develop progressive CNS symptoms, e.g. mental retardation, seizures, spasticity, ataxia and intention tremor. In addition, some

patients suffer from recurrent severe bacterial infections, which is probably due to defective granulocyte function.

Ophthalmological abnormalities, e.g. fundus lesions, retinal pigmentations, crystalline opacities in the lenses, poor adaptation to darkness and pathological electroretinograms, have been described in some patients [3]. Retinal dystrophy has also been observed in adult patients [4]. Antenatal cerebral haemorrhage has been reported in a patient with moderate GS deficiency [5], and two further cases of cerebral haemorrhages in two neonates were observed in post-mortem investigations [2]. 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 [6].

It is estimated that about 25% of all patients with GS deficiency die early, often in the neonatal period, because of electrolyte imbalances and/or infections [7].

The first pregnancy in a woman with moderate GS deficiency has been reported to be uneventful, resulting in an unaffected infant [8].

▪ Metabolic Derangement

As the enzyme catalyses the last step of GSH synthesis, its deficiency leads to low cellular concentrations of GSH and excessive production of the dipeptide 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 the action of γ-glutamyl cyclotransferase. The excessive formation of 5-oxoproline exceeds the capacity of 5-oxoprolinase, leading to accumulation of 5-oxoproline in body fluids causing metabolic acidosis and 5-oxoprolinuria. γ-Glutamylcysteine contains both reactive groups of GSH (i.e. the γ-glutamyl and the sulfhydryl residues). It accumulates in fibroblasts of patients with GS deficiency in levels similar to that of GSH in control cells and may to some extent compensate for lack of GSH. GSH also takes part in the synthesis of leukotriene C_4, the primary cysteinyl leukotriene. It has been shown that the synthesis of lipoxygenase products is impaired in patients with GS deficiency.

▪ 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. About 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 [9]. 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 [9]. 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 skin fibroblasts and decreased activity of GS in erythrocytes and/or cultured skin fibroblasts. Enzyme activities of 1-30% of healthy controls are found in affected patients. Mutation analysis confirms the diagnosis. One symptomatic patient with GS deficiency has been identified through tandem mass spectrometry-based newborn screening. Antenatal diagnosis can be performed by mutation analysis of chorionic villi, analysis of GS activity in cultured amniocytes or chorionic villi, or by measuring the levels of 5-oxoproline in amniotic fluid.

▪ Treatment and Prognosis

The clinical management of GS deficient patients is aimed at correction of acidosis, prevention of haemolytic crises and support of endogenous defence against reactive oxygen species (ROS). In the neonatal period, correction of metabolic acidosis, electrolyte imbalances, treatment of anaemia and excessive hyperbilirubinaemia are of crucial importance.

Correction of acidosis can be reached through bicarbonate, citrate or tris-hydroxymethyl aminomethane (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 haemolysis. Drugs and foods known to precipitate haemolytic crises in patients with 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 in GS-deficient patients [2].

The value of *N*-acetylcysteine, which is known to protect cells from oxidative stress in vitro, in the treatment of GS deficiency is controversial. It was suggested that the low intracellular GSH concentrations and cysteine availabilty might be increased by *N*-acetylcysteine. However, 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 two patients with GS deficiency [10]. 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 normalised intracellular GSH content [11].

Owing to the rarity of the disease and the heterogeneity of the clinical condition the prognosis for individual patients is difficult to predict. Early diagnosis, correction of acidosis and early supplementation with vitamin E and vitamin C appear to be the most important factors regarding the survival and the long-term outcome.

30.1.3 γ-Glutamyl Transpeptidase Deficiency

▪ Clinical Presentation

Up to now, seven patients in five families have been reported worldwide. Five of these patients were characterised by central nervous system involvement. However, two affected siblings presented without any signs of CNS involvement at the ages of 11 and 13 years [12]. 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 facing the external side of the cell. 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. Detailed studies in three patients with γ-glutamyl transpeptidase deficiency have demonstrated that they also have a complete deficiency of leukotriene D_4 biosynthesis.

Knock-out mice with γ-glutamyl tramnspeptidase deficiency present with glutathionuria, glutathionaemia, growth failure, cataracts, lethargy, shortened life span, and infertility [13].

▪ 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 which are located on the long arm of chromosome 22. So far, no mutations have been identified in patients with γ-glutamyl transpeptidase deficiency.

▪ Diagnostic Tests

High levels of GSH are present in plasma and urine (up to 1 g/day in urine; controls <10 mg) of all patients, whereas cellular levels of GSH are normal. Decreased activity of γ-glutamyl transpeptidase is found in nucleated cells such as leukocytes or cultured skin fibroblasts. Erythrocytes are not useful for diagnostic purposes, since they also lack γ-glutamyl transpeptidase under normal conditions.

▪ Treatment and Prognosis

There is no specific treatment or management for a better long-term prognosis. However, administration of *N*-acetylcysteine to γ-glutamyl transpeptidase-deficient mice for 2 weeks led to restoration of their fertility [13].

30.1.4 5-Oxoprolinase Deficiency

▪ Clinical Presentation

Up to now, eight patients in six different families have been described. The clinical symptoms are inconstant and very heterogeneous, including renal stone formation, enterocolitis, neonatal hypoglycaemia, microcytic anaemia, microcephaly and mental retardation. It remains to be established wheter symptoms in identified patients are merely a coincidence.

▪ 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. In mammalians the enzyme is not well studied. Apparently it is composed of two identical 142-kDa subunits. Decreased activity leads to decreased conversion of 5-oxoproline to glutamate, resulting in levated levels of 5-oxoproline in body fluids.

■ **Genetics**

The mode of inheritance is autosomal recessive. The location of the corresponding gene in the human genome remains to be established.

■ **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. Decreased acitivity of 5-oxoprolinase in nucleated cells (e.g. leukocytes or skin fibroblasts) are decreased. Erythrocytes are not a suitable diagnotic tool because the enzyme is not even present under normal conditions.

■ **Treatment and Prognosis**

No specific treatment has bee proposed or tried. Prognosis remains to be established.

30.1.5 Dipeptidase Deficiency

■ **Clinical Presentation**

So far, dipeptidase deficiency has been suggested in only one patient worlwide [14]. The 15-year-old boy presented with mental retardation, mild motor impairment, and partial deafness.

■ **Metabolic Derangement**

Dipeptidase is a membrane-bound enzyme that hydolyses dipeptides, including cysteinylglycine compounds, such as the oxidised γ-glutamyl transpeptidase product cystinyl-bis-glycine and the conversion of leukotriene D_4 to E_4. This leads to cystinylglycinuria and increased excretion of leukotriene D_4.

■ **Genetics**

The crystal structure of human membrane-bound dipeptidase has been reported. This enzyme is 42 kDa while underglycosylated and 63 kDa when glycosylated. Renal dipeptidase has been mapped to human chromosome 16 at q24. No genetic studies have been performed in the only patient known so far.

■ **Diagnostic Tests**

Diagnosis in the described patient was based on the finding of increased urinary excretion of cystinylglycine and leukotriene D_4, which is usually not detectable. Leukotriene E_4, the major urinary metabolite in humans, was completely absent. The diagnosis has not been confirmed by enzyme analysis in this patient with suspected dipeptidase deficiency.

■ **Treatment and Prognosis**

No specific treatment has been proposed or tried. Prognosis remains to be established.

30.1.6 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 that should be considered during diagnostic work [15]. Excessive formation of 5-oxoproline has been described (e.g.) in patients with urea cycle defects, such as ornithine transcarbamoylase deficiency or homocystinuria. In nephropathic cystinosis 5-oxoprolinuria may occur because of secondary impairment of the γ-glutamyl cycle resulting from decreased availability of free cysteine and can be corrected through cysteamine therapy. Transient 5-oxoprolinuria of unknown cause has been reported in very preterm infants. 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, such as paracetamol, vigabatrin or some antibiotics (flucloxacillin, netimicin), are known to induce 5-oxoprolinuria, probably through interaction with the γ-glutamyl cycle. Particular infant formulas and tomato juice may contain modified proteins with increased content of 5-oxoproline.

30.2 Disorders of Imidazole Dipeptides

30.2.1 Serum Carnosinase Deficiency

■ **Clinical Presentation**

Some 30 individuals have been reported with this disorder, first described in 1967 [16, 17]. The majority of them showed mental retardation to a variable degree. Some patients had seizures, and one had congenital myopathy. A few had no symptoms at all, making the relationship between the biochemical abnormalities and the clinical picture uncertain [18].

■ **Metabolic Derangement**

The deficiency of serum carnosinase activity causes persistent carnosinuria during a meat-free diet. Several variants with abnormal kinetic properties of the enzyme have been described. In the cerebrospinal fluid (CSF) of affected persons, homocarnosine can be normal or increased.

■ **Genetics**

Inheritance is autosomal recessive. Serum carnosinase deficiency was reported in a child with 18q syndrome, suggesting a chromosomal location of this enzyme [19].

■ **Diagnostic Tests**

The diagnosis is made by quantitative amino acid analysis of serum and/or urine after exclusion of meat from the diet. Anserine appears in the urine of these persons only after eating food containing the dipeptide. Normal persons excrete 1-methylhistidine after ingesting anserine; in serum carnosinase deficiency, there is little or no 1-methylhistidine excretion. The diagnosis is confirmed by measuring carnosinase activity in serum. It has to be noted that serum carnosinase activity may be low in other disorders, such as urea-cycle disorders and multiple sclerosis [20].

■ **Treatment and Prognosis**

No efficient treatment is available. In view of the above remarks, it is uncertain whether treatment would be necessary. There is no reason to withhold meat from the diet, because the accumulating carnosine is primarily endogenous. Prognosis is variable and does not seem to correlate with the degree of enzyme deficiency.

30.2.2 Homocarnosinosis

■ **Clinical Presentation**

This condition was described in 1976 in a Norwegian family (three of four siblings and their mother) [21], and in 2001 in a Russian boy and his father [22]. The three Norwegian siblings showed progressive spastic diplegia, mental retardation and retinitis pigmentosa, with onset between 6 and 29 years of age. Their mother, however, was symptom free. The Russian boy showed moderate psychomotor retardation and hypotonia. His father was symptom free. This makes it uncertain whether there is a relationship between the biochemical defect and the clinical symptoms.

■ **Metabolic Derangement and Diagnostic Tests**

In the CSF of the three siblings and in that of their clinically normal mother, the homocarnosine level was 30-50 times the mean of control levels. The carnosine levels were normal. Deficiency of homocarnosinase activity was found [23]. Therefore, serum carnosinase deficiency and homocarnosinosis are probably the same disorder. The diagnosis is made by quantitative amino acid analysis of the CSF.

■ **Genetics**

Inheritance in the two reported families seems to be autosomal dominant.

■ **Treatment and Prognosis**

The remarks regarding treatment and prognosis of serum carnosinase deficiency also apply here.

30.2.3 Prolidase Deficiency

■ **Clinical Presentation**

Some 80 individuals with prolidase deficiency have been reported since 1968 [24, 25]. About a quarter of them were asymptomatic at the time of the report. The others had their first symptoms between birth and 22 years of age. All patients showed skin lesions, either mild (face, palms, soles) or severe, and had recalcitrant ulceration, particularly on the lower legs. Other features included a characteristic face, impaired motor or cognitive development, recurrent infections, and chronic lung disease resembling cystic fibrosis [25]. Prolidase deficiency seems to be a risk factor for the development of systemic lupus erythematosus. Alternatively, patients with systemic lupus erythematosus should, where there is a family history or presentation in childhood, be specifically investigated for prolidase deficiency [25, 26].

■ **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. The gene *(PEPD)* maps to chromosome 19p13.2. Some 19 mutations have been identified [25, 27, 28].

■ **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 [29]. As to prognosis, the age at onset and the severity of clinical expression are highly variable. Studies are under way to deliver liposome-encapsulated prolidase intracellularly [30].

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Trimethylaminuria and Dimethylglycine Dehydrogenase Deficiency

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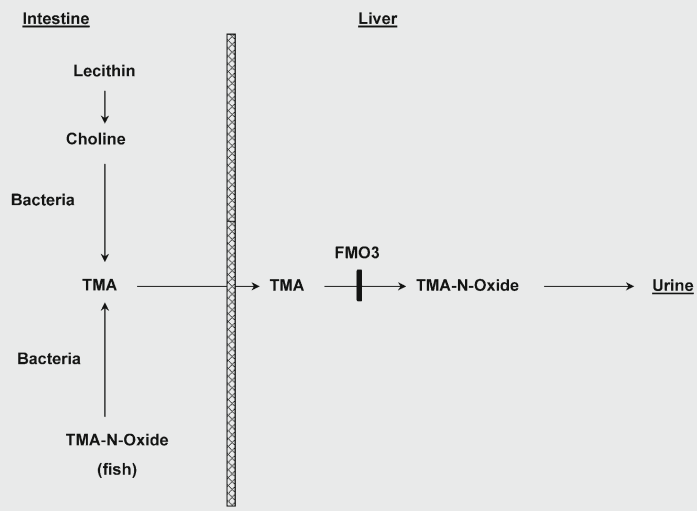
J.-M. Saudubray et. al (Eds.), *Inborn Metabolic Diseases,* DOI 10.1007/978-3-642-15720-2_31,

Trimethylamine Metabolism

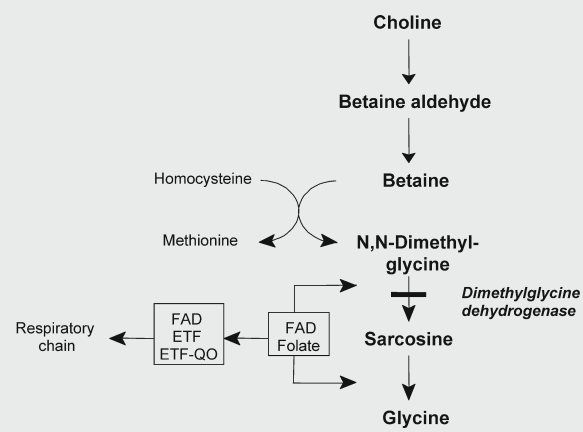
In man, trimethylamine (TMA) has a dietary origin. It is produced in the intestine by a bacterial action on food, mainly choline (in lecithin), TMA *N*-oxide, present in saltwater fish and shellfish, and possibly carnitine. TMA is absorbed from the gut, transported to the liver and oxidised by flavin-containing monooxygenase 3 (FMO3) to TMA *N*-oxide, a nonodorous product which is excreted in urine (◘ Fig. 31.1). On an average Western diet, the combined urinary excretion of TMA and TMA *N*-oxide of normal adults is around 50 mg per day, with less than 10% as TMA [1-3]. FMO3 is one of five human microsomal FMOs and is the main FMO in adult liver. It has a single flavin adenine dinucleotide (FAD) and catalyses NADPH-linked oxidation of other endogenous amines, (*s*)-nicotine, and many exogenous compounds and drugs, including tamoxifen, cimetidine, ranitidine, ketoconazole, clozapine, amphetamine and cysteamine [3-7]. Enzyme activity is inhibited by indoles from brassicas and is reduced perimenstrually [2, 8]. The major FMO in fetal liver is FMO1. After birth there is a switch to FMO3. The onset of expression varies. Few infants produce this isoform neonatally, but most produce significant amounts by the age of 1-2 years [9].

Catabolism of Choline

This process (◘ Fig. 31.2) 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. 31.1.** Metabolism of trimethylamine. *FMO3*, flavin-containing monooxygenase 3; *TMA*, trimethylamine. The enzyme defect in trimethylaminuria is indicated by a *solid bar*



◘ **Fig. 31.2.** 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*

These disorders cause an unpleasant fish-like body odour. The problems are psychosocial and management centres on attempting to minimise the odour.

31.1 Trimethylaminuria (Fish Odour Syndrome)

31.1.1 Clinical Presentation

Individuals homozygous for trimethylaminuria present with an offensive body odour, usually in childhood, but sometimes as adults. Subjects may be unaware of their own odour. *Transient* trimethylaminuria has been reported in infancy and is perhaps due to delayed FMO3 expression. The severity is variable. The odour may be episodic, becoming apparent with sweating and menstruation, and after a high dietary intake of TMA precursors [2, 10]. In two affected breast-fed babies, the smell occurred after their mother ate eggs or fish [11]. There are no physical abnormalities. Heterozygotes do not have the odour normally, but it may appear after a TMA load [1].

The disorder often causes psychosocial problems, with anxiety, low self-esteem, social isolation and poor school performance [10, 12]. FMO3 deficiency may have contributed to reported adverse reactions to tyramine, sulfur-containing medications (1 patient) and labile hypertension [5].

31.1.2 Metabolic Derangement

Trimethylaminuria results when the oxidative capacity of FMO3 is overwhelmed by TMA substrate. This is most often caused by a deficiency of FMO3 (primary trimethylaminuria) attributable to mutations of the *FMO3* gene or reduced enzyme expression (in immaturity and perhaps perimenstrually). Alternatively, high choline or TMA *N*-oxide intake and/or an abnormal gut flora may produce excess TMA (secondary trimethylaminuria). Malodorous TMA accumulates in urine, sweat, saliva, breath and vaginal secretions and, being volatile, is released readily into the atmosphere. In primary trimethylaminuria, TMA accounts for more than 10% of the combined TMA/TMA *N*-oxide urinary excretion. In secondary trimethylaminuria, both TMA and TMA *N*-oxide excretion are increased, with TMA usually less than 10% of the total [2, 3, 10].

31.1.3 Genetics

Trimethylaminuria is an autosomal recessive disorder [10, 13] with an estimated carrier frequency in the United Kingdom of 1% [2]. The *FMO3* gene is located in a cluster of six *FMO* genes on chromosome 1 (q23-q25) [7, 14]. Missense and nonsense mutations have been identified which cause significant reduction of FMO3 activity in vitro. Two common ones are P153L and E305X. M66I and R492W mutations prevent binding/retention of FAD [15]. Homozygosity or compound heterozygosity for these loss-of-function mutations is associated with moderate or severe trimethylaminuria. In addition, at least eight gene polymorphisms associated with normal or only slightly decreased enzyme activity are known. These may not present clinically under normal circumstances, but may be associated with trimethylaminuria when challenged by excessive TMA, or when enzyme activity is reduced at menstruation, accounting for some cases of transient or apparent secondary trimethylaminuria [5, 6, 16-18].

31.1.4 Diagnostic Tests

Common causes of abnormal body odour and urine infection are excluded. A random urine sample is collected when the patient is on a normal diet and not menstruating, and preferably when the odour is present. The sample is acidified quickly to pH 2.0 and frozen until analysis by NMR spectroscopy or headspace gas chromatography-mass spectrometry. A ratio of TMA to total TMA of 10-39% is consistent with mild, and one of 40% and over with severe, trimethylaminuria. Gene sequencing should be offered [3]. A ratio less than 10% with *raised* TMA (> 20 μmol/mmol creatinine) may indicate secondary trimethylaminuria. The test is repeated, again while the patient is on a normal diet, after completion of a 1-week course of metronidazole or neomycin to alter the gut microflora. A ratio of less than 10% with *normal* TMA excretion may be found in unaffected individuals, heterozygotes or those with intermittent trimethylaminuria. An oral challenge with choline bitartrate, if available (2.5-15 g according to age), or a meal of fish [19] or of eggs with baked beans should unmask latent trimethylaminuria. An oral TMA-loading test will detect heterozygotes [10, 13], but genetic testing is simpler in families with a known mutation.

31.1.5 Treatment

Explanation of the problem provides insight. Dietary restrictions may reduce the odour. Foods to avoid include eggs, mayonnaise, liver, kidney, peas, beans, peanuts, soya products, lecithin-containing health supplements, seafish, shellfish, squid, octopus and cuttlefish, and also brassicas (brussels sprouts, broccoli, cabbage, cauliflower). Choline

intake should be adequate for health [20] and should not be restricted during pregnancy and lactation. Folate requirement is increased, necessitating a good dietary intake. Riboflavin supplements may enhance residual FMO3 activity. Appropriate clothing and room ventilation minimise sweating, and the use of acid soap or body lotions (pH 5.5-6.5) traps TMA as nonvolatile salts. Short courses of copper chlorophyllin or activated charcoal to sequester TMA in the gut may help [21]. Long-term use of antibiotics to reduce enteric TMA production should be avoided, but a 2-week course of metronidazole (250 mg three times daily) or short courses of lactulose may provide some protection for special occasions and holidays [2, 3, 12].

31.2 Dimethylglycine Dehydrogenase Deficiency

31.2.1 Clinical Presentation

Up to now, only one case has been reported. This male patient was investigated at the age of 38 years for an abnormal body odour resembling fish, which had been present since he was 5 years of age, was increased by stress and effort and caused him major social, psychological and professional problems. He also had chronic muscle fatigue with persistent elevation of creatine kinase to around 4 times normal. He had normal intelligence. His siblings and two sons were asymptomatic [22].

31.2.2 Metabolic Derangement

Dimethylglycine dehydrogenase (DMGDH) deficiency blocks choline catabolism. This caused an accumulation of around 100-fold normal in the patient's plasma and of around 20-fold in his urine of volatile *N,N*-dimethylglycine, and an unpleasant fish odour [22].

31.2.3 Genetics

The pedigree of the patient with DMGDH deficiency suggests autosomal recessive inheritance. The *DMGDH* gene is on chromosome 5q12.2-12.3 [23]. Sequence analysis suggests that the genes for DMGDH and SDH have diverged from a common ancestor. The affected patient is homozygous for a point mutation (A326G) of the *DMDGH* gene. From expression studies, this mutated gene codes for a stable protein lacking enzyme activity [24]. The mutated enzyme has a 27-fold decrease in specific activity and a 65-fold increase in K_m, explaining the pathogenicity of the mutation [25].

31.2.4 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 [22]. Dimethylglycine is not detected with gas chromatography-mass spectrometry procedures using solvent extraction, which are 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
	All over 2 months of age (children and adults)	<26 mmol/mol creatinine

Increased serum levels have been observed in folate deficiency (up to 10-fold), cobalamin deficiency (up to 2-fold) and renal failure (up to 2-fold) [22].

DMGDH activity is not normally detectable in blood cells and fibroblasts; a liver biopsy would be necessary to confirm low enzyme activity, but is invasive and probably unnecessary in this disorder.

31.2.5 Treatment

Management is by counselling and by 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 alone, but a trial of folate with riboflavin was suggested [22].

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VII Disorders of Lipid and Bile Acid Metabolism

Dyslipidaemias

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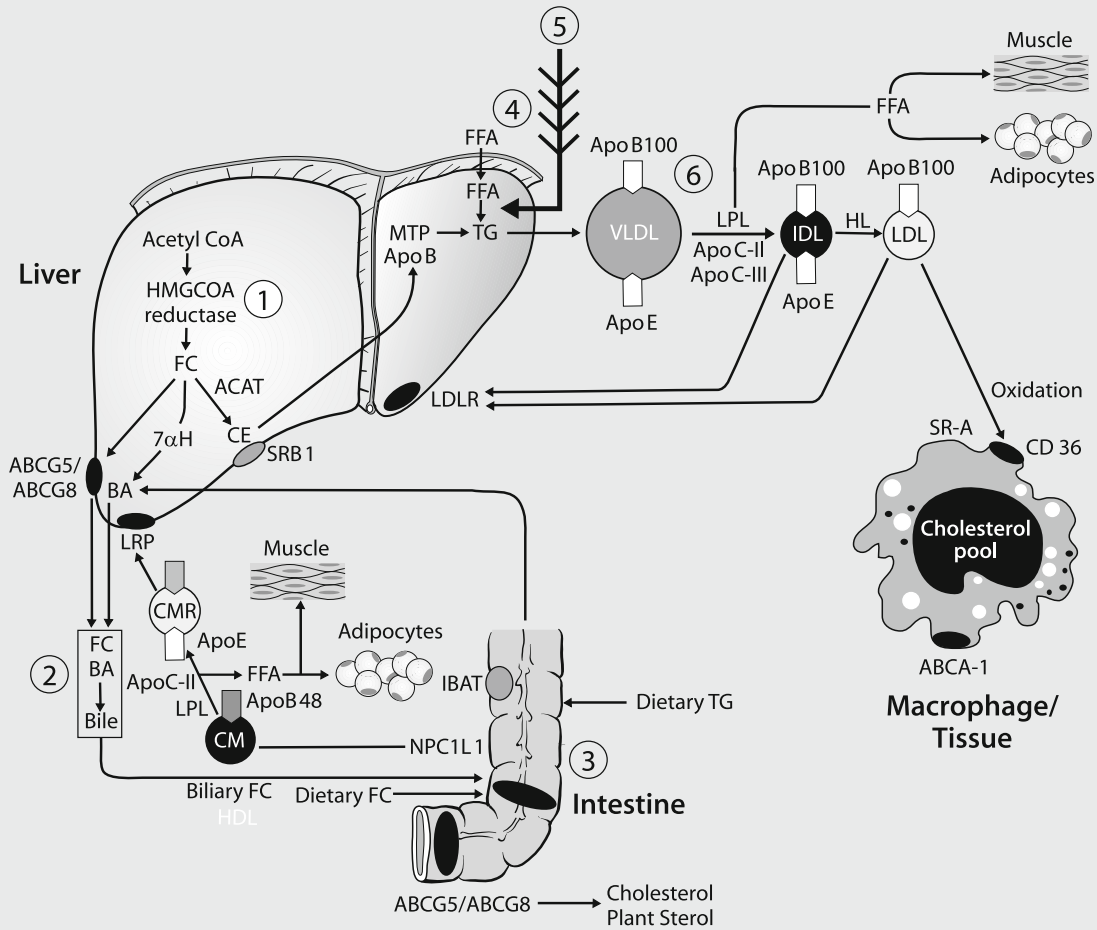
Llipoprotein metabolism

Human plasma lipoprotein metabolism appears complex, but can be dissected into three major pathways: the exogenous (intestinal) pathway; the endogenous (hepatic) pathway and reverse cholesterol transport (◘ Fig. 32.1, Fig. 32.2). An understanding of normal lipid, apolipoprotein and lipoprotein metabolism is particularly important in order to make the correct choice of lipid-altering agents and to interpret the efficacy of treatment, the major goals being to prevent cardiovascular disease (CVD) and pancreatitis.



◘ **Fig. 32.1.** Pathways of exogenous (intestinal) and endogenous (hepatic) lipoprotein metabolism. Chylomicrons (*CM*) transport lipids of dietary and hepatic origin. Cholesteryl esters (*CE*) and triglycerides (*TG*) are emulsified by bile acids (*BA*), hydrolysed by pancreatic lipases, absorbed by the small intestine, resynthesised and packaged by microsomal TG transport protein (*MTP*) and apoB-48 into CM, which are then secreted. TG in CM are hydrolysed by lipoprotein lipase (*LPL*) and apoC-II, producing free fatty acids (*FFA*), which can be taken up by adipocytes or muscle. The CM remnant (*CMR*) is then taken by the low-density lipoprotein-like receptor (*LRP*) in liver. BA are reabsorbed through the ileal bile acid transporter (*IBAT*) and recycled to the liver. Free cholesterol (*FC*) is synthesised in the liver through HMGCoA reductase and can be excreted from the liver into bile by ATP-binding cassette (*ABC*) proteins G5 or G8. FC can also be converted into BA by 7 ά hydroxylase (*7áH*), or esterified by acyl cholesterol acyltransferase (*ACAT*) into CE. CE interacts with apoB-100, reducing its proteolysis, and TG is added by MTP, producing very low-density lipoprotein (*VLDL*), which contains one molecule of ApoB-100 that is required for its secretion. The TG on VLDL are hydrolysed by LPL and apoC-II, producing FFA and IDL. Some IDL is removed by the interaction of apoE with the hepatic LDL receptor (*LDLR*), while the rest is converted into LDL by hepatic lipase (*HL*). ApoC-III inhibits both LPL and apoE-mediated IDL uptake. LDL is normally removed by the LDLR; excess LDL can be oxidised in the vascular wall and taken up by the scavenger receptors, CD 36 and SR-A, on macrophages, promoting CE storage. The sites of actions of the six major classes of drugs are depicted as follows: **1**, HMG-CoA reductase inhibitors; **2**, bile acid sequestrants; **3**, cholesterol absorption inhibitors; **4**, niacin; **5**, omega-3 fish oils; **6**, fibric acid derivatives. (Reproduced with permission from [63])

Conversion Factors		
mg/dl → mmol/l → mg/dl		
Cholesterol	x 0.0259	x 38.6
TG	x 0.0114	x 87.7
Phospholipids	x 0.323	x 77.5

32.1 Overview of Plasma Lipid and Lipoprotein Metabolism

The lipoprotein structure resembles a plasma membrane bilayer with hydrophilic phospholipids (PL), apolipoproteins and some unesterified cholesterol on the outer surface, and hydrophobic triglycerides (TG) and cholesteryl esters (CE) in the core [1]. The major human plasma lipoproteins can be classified according to their hydrated density, electrophoretic mobility or chemical composition (◘ Table 32.1). Chylomicrons and very low-density lipoproteins (VLDL) are the major carriers of TG, while low-density lipoproteins (LDL) and high-density lipoproteins (HDL) transport most of the CE.

The major lipoproteins have a variety of apolipoproteins associated with them (◘ Table 32.2), each of which has one or more functions, such as ligands for lipoprotein receptors, co-factors for enzymes, and structural proteins for packaging lipoproteins. The nomenclature for apoli-

◘ **Table 32.1.** Physical-chemical properties of human plasma lipoproteins

Class	Density (g/ml)	Electrophoretic mobility	Surface components			Core lipids	
			Cholesterol	Phospholipids	Apolipoproteins	TG	Cholesteryl esters
Chylomicrons	<0.95	Remains at origin	2	7	2	86	3
VLDL	0.950-1.006	Pre-β-lipoproteins	7	18	8	55	12
IDL	1.006-1.019	Slow pre-β-lipoproteins	9	19	19	23	29
LDL	1.019-1.063	β-Lipoproteins	8	22	22	6	42
HDL-2[1]	1.063-1.125	α-Lipoproteins	5	33	40	5	17
HDL-3[1]	1.125-1.210	α-Lipoproteins	4	35	55	3	13
Lp(a)[2]	1.040-1.090	Slow pre-β-lipoproteins					

VLDL, very low-density lipoprotein; *IDL*, intermediate-density lipoprotein; *LDL*, low-density lipoprotein; *HDL*, high-density lipoprotein; *TG*, triglycerides. [1]HDL-2 and HDL-3 are the two major subclasses of HDL. [2]Lp(a) consists of a molecule of LDL covalently attached to a molecule of apo(a), a glycoprotein homologous with plasminogen. Its lipid composition is similar to that of LDL. Compositions are given as percentages (by weight)

◘ **Table 32.2.** Characteristics of major human plasma apolipoproteins

Apolipoproteins	Major tissue sources	Functions	Molecular weight
Apo A-I	Liver and intestine	Removes cell cholesterol via ABCA1 onto nascent HDL; co-factor LCAT; facilitates uptake of cholesteryl esters from HDL, LDL and VLDL by SR-B1	29,016
Apo A-II		Not known	17,414
Apo A-IV		Activates LCAT; helps form chylomicrons	44,465
ApoA-V		Stimulates proteoglycan-bound LPL	39,000
Apo B-48	Intestine	Secretion of chylomicrons from intestine	240,800
Apo B-100	Liver	Secretion of VLDL from liver; binding ligand of LDL to LDLR	512,723
Apo C-I	Liver	Activates LCAT; inhibits CETP and SR-B1	6,630
Apo C-II		Co-factor LPL	8,900
Apo C-III		Inhibits LPL and binding of IDL to LDLR	8,800
Apo D	Many sources	Promotes reverse cholesterol transport	19,000
Apo E	Liver	Ligand for uptake of chylomicron remnants and IDL by LRP and LDLR	34,145

ABCA1, ATP-binding cassette transporter 1; *LCAT*, lecithin cholesterol acyl transferase; HDL, high-density lipoproteins; *LDL*, low-density lipoproteins; *VLDL*, very low-density lipoproteins; *SR-BI*, scavenger class B type I receptor; *LPL*, lipoprotein lipase; *CETP*, cholesteryl ester transfer protein; *IDL*, intermediate-density lipoprotein; *LDLR*, low-density lipoprotein receptor; *LRP*, low-density lipoprotein-like receptor protein

poproteins is alphabetical (■ Table 32.2). Apolipoprotein A-I (apoA-I) constitutes about 70% of the apolipoproteins of HDL. Apolipoprotein B (apoB) is the major apolipoprotein of chylomicrons VLDL and LDL, and of the lipoprotein particles that result from the hydrolysis of TG, namely, chylomicron remnants, VLDL remnants and intermediate density lipoproteins (IDL) (1.006-1.019 g/ml). ApoB is also a major component of lipoprotein(a), or Lp(a) lipoprotein. The full-length apoB polypeptide, apoB-100, is made in liver, and one molecule of apoB is found in VLDL, VLDL remnants, IDL, LDL and Lp(a). A truncated version of apoB, apoB-48, a product of the same gene as apoB-100, is shortened due to post-translational modification. ApoB-48 is made in intestine and is found in chylomicrons and chylomicron remnants (■ Table 32.2). The entire sequence of the gene for each apolipoprotein listed in ■ Table 32.2 is known (www.ncbi.nlm.nih.gov/sites/entrez).

32.1.1 Exogenous Lipoprotein Metabolism

The exogenous pathway of lipoprotein metabolism transports dietary fats from intestine to muscle, adipose tissue and liver (■ Fig. 32.1). Dietary lipids, mainly TG, and PL, are emulsified by bile acids and hydrolysed by pancreatic lipases into their component parts, monoglyceride, free fatty acids (FFA) and unesterified cholesterol. After absorption into the intestinal cells, the FFA and monoglycerides are synthesised into TG and incorporated with cholesterol into chylomicrons (CM) by the microsomal triglyceride transport protein (MCT). CM contain apolipoproteins A-I, A-IV, and B-48 [2] (■ Table 32.2). About 90% of the lipid in CM is TG (■ Table 32.1). CM are secreted into the thoracic duct, a process that requires apoB-48. Thereafter, CM enter the peripheral circulation, where they acquire apoE and apoC-I, apoC-II and apoC-III from HDL. When CM enter the capillaries of skeletal muscle and adipose tissue, they are exposed to the enzyme lipoprotein lipase (LPL), which is attached to the surface of the endothelial cells (■ Table 32.3). LPL along with its co-factor, apoC-II, hydrolyse TG to FFA, which then enter muscle and adipose tissue (■ Fig. 32.1). Chylomicron remnants (CMR), containing TG, cholesterol, apoB-48 and apoE, are taken up into the liver by a receptor-mediated endocytosis involving low-density lipoprotein-like receptor protein (LRP) [3] (■ Fig. 32.1). In the liver cholesterol is used for lipoprotein synthesis or cell membrane structure, or excreted into bile, either as unesterified (free) cholesterol (FC) or as bile acids derived from cholesterol. The cholesterol on the CMR can be derived from diet or liver (■ Fig. 32.1, left lower part).

32.1.2 Endogenous Lipoprotein Metabolism

The endogenous pathway of lipoprotein metabolism involves the hepatic production of the TG-rich VLDL (■ Fig. 32.1). The major apolipoproteins of VLDL are apoB-100, apoE, and apoC (I, II, III) (■ Table 32.2).

Biosynthesis of VLDL. In the fasting state, most of the plasma TG is carried by VLDL. TG, CE and apoB-100 in

■ **Table 32.3.** Key enzymes and transfer proteins of plasma lipid transport

Enzyme	Major tissue source	Functions	Molecular weight
Lipoprotein lipase (LPL)	Adipose tissue Striated muscle	Hydrolyses TG and phospholipids of chylomicrons and large VLDL	50,394
Hepatic lipase (HL)	Liver	Hydrolyses TG and phospholipids of small VLDL, IDL, and HDL-2	53,222
Lecithin:cholesterol acyltransferase (LCAT)	Liver	Transfers a free fatty acid from phosphatidylcholine on nascent (pre-β) HDL to form cholesteryl esters and mature HDL	47,090
Cholesterol ester transport protein (CETP)	Liver, spleen and adipose tissue	Transfers cholesteryl esters from HDL to apoB-containing lipoproteins Converts α-HDL to pre-β-HDL	74,000
Phospholipid transfer protein (PTP)	Placenta, pancreas, adipose tissue, lung	Transfers the majority of phospholipids in plasma Converts α-HDL to pre-β-HDL	81,000
Scavenger class B type I receptor	Liver, adrenal, gonads, endothelium, macrophages	Mediates the selective uptake of cholesteryl ester from the core of lipoproteins, including HDL, LDL and VLDL	82,000

liver are required for VLDL synthesis. Hepatic FFA are normally activated (fatty acid CoA) and then oxidised, or incorporated into TG or CE. When there is increased flux of FFA to liver, the ability of the oxidative or storage pathways to metabolise fatty acid CoA is exceeded. Intermediates of fatty acid metabolism accumulate and can both stimulate TG formation and activate serine kinases, which negatively regulate insulin action.This latter inhibitory effect on insulin action increases cholesterol synthesis and stimulates apoB secretion.

ApoB-100 is made constitutively in liver. The expression of *APOB* is not regulated. The quantity of apoB-100 in liver is regulated by proteolysis. Only some of the apoB-100 molecules that are synthesised survive to become incorporated into VLDL; the remainder are degraded by proteolytic enzymes. When CE interacts with apoB-100, it is less likely to be degraded, leading to increased production of apoB-100. MTP catalyses the incorporation of TG into this complex, producing VLDL (◘ Fig. 32.1, left upper part).

Secretion and Metabolism of VLDL. ApoB-100 is necessary for the secretion of VLDL into plasma. VLDL-TG are transported to tissue capillaries, where they are hydrolysed by LPL and apoC-II, thereby releasing FFA. The TG in the resulting large VLDL remnants is further hydrolysed, generating intermediate-density lipoproteins (IDL) (◘ Table 32.1). A portion of IDL is cleared from the circulation via direct hepatic uptake through the binding of apoE on IDL to the LDL receptor (LDLR) (◘ Fig. 32.1). The rest of the IDL undergo further hydrolysis by hepatic lipase (HL) (◘ Table 32.3), producing the final end-product of this metabolic cascade, namely, LDL (◘ Fig. 32.1, central upper part).

LDL Binding and Internalisation. The LDLR pathway was elegantly discovered by Goldstein and Brown and their group [4]. The LDLR is synthesised in the endoplasmic reticulum (ER). After glycosylation in the ER and Golgi, LDLR is directed toward clathrin-coated pits, where the ligand-binding domain of the LDLR is exposed to apoB-100. This receptor-ligand complex is internalised within coated vesicles by endocytosis and delivered to endosomes with the help of an adaptor protein, called autosomal recessive hypercholesterolaemia (ARH) [5]. The apoB-100 moiety on LDL and the apoE ligand on TG-rich lipoproteins both bind to the LDLR with high affinity, promoting their internalisation and cellular metabolism (◘ Fig. 32.1, central upper part).

LDL Degradation. In the acidic environment of the endosomes, LDL is displaced from the LDLR, permitting release of LDL into the endosomes and recycling of the LDLR to the cell surface [4, 5]. The released LDL is subsequently degraded in the lysosome [5].

Regulatory Effect of Cholesterol Derived from LDL Degradation. In lysosomes the apoB-100 of LDL undergoes proteolysis and CE are hydrolysed into FC and FFA. The FC derived from LDL decreases both HMG-CoA reductase and LDLR activity by inhibiting the sterol regulatory element-binding protein (SREBP) pathway. Cholesterol regulates the proteolytic release of SREBPs, a family of transcription factors, from the ER [4, 6]. This effect occurs through the SREBP cleavage-activating protein (SCAP), which is both a sensor of sterols and an escort of SREBPs. As the cholesterol content of the hepatocytes decreases, the transcription of the LDLR and HMG-CoA reductase genes increases, and vice versa [4, 6].

Through LDLR, LDL also supplies cholesterol to extrahepatic parenchymal tissues, for membrane synthesis or as a precursor for steroid and sex hormone synthesis. LDL can also be removed via non-LDLR mechanisms by scavenger receptors such as SR-A, CD36, and scavenger receptor class B type I (SR-BI), which take up chemically modified oxidised or glycated LDL (◘ Fig. 32.1, right part). Scavenger receptors are not regulated by intracellular cholesterol levels. In peripheral tissues, such as macrophages, excess cholesterol accumulates within the plasma membrane and is esterified to CE by the enzyme acyl-CoA cholesterol acyltransferase (ACAT). Cytoplasmic lipid droplets are then formed, and the cells are then converted to foam cells (an early stage of atherogenesis) (◘ Fig. 32.1).

The optimal level of LDL to prevent atherosclerosis and maintain normal cholesterol homeostasis in humans is not known. At birth, the average LDL-C level is about 30 mg/dl. After birth, if the LDL-C is <100 mg/dl, LDL is mostly removed through the high-affinity LDLR pathway. In Western societies, LDL-C is usually >100 mg/dl; the higher the LDL-C, the greater the amount that is removed by the scavenger pathway.

While the exogenous and endogenous pathways are conceptually considered as separate pathways, an imbalance in one often produces an abnormal effect in the other (◘ Fig. 32.1). Thus, reduced LPL activity or decreased apo C-II, and also elevated apo C-III or apo C-I, can promote hypertriglyceridaemia and accumulation of remnant particles from both chylomicrons and VLDL.

32.1.3 Reverse Cholesterol Transport and High-density Lipoproteins

Reverse cholesterol transport (◘ Fig. 32.2) refers to the process by which FC is removed from extrahepatic tissues,

probably by extraction from cell membranes by lipid-poor apoA-I via the ATP binding cassette proteins, ABCA1, or by ABCG1 or SR-BI (Table 32.3) to a mature HDL particle and transported back to the liver for disposal [7, 8].

Synthesis of HDL

Nascent HDL. ApoA-I is released as a lipid-free protein from intestinal and liver cells (Fig. 32.2). ApoA-I interacts with ABCA1 on the basolateral membranes of hepatocytes, enterocytes and macrophages, acquiring PL and FC to form a more stable nascent HDL particle [7, 8] (Fig. 32.2).

Formation of Larger, Mature HDL Particles. Transformation of HDL particles to the spherical 'mature' HDL requires the esterification of cholesterol by LCAT and its co-factor, apoA-1, to create CE for the hydrophobic core (Fig. 32.2). The subsequent addition of cellular cholesterol to the HDL particle occurs in macrophages and other peripheral cells through the action of ABCG1 and SR-BI, molecules that prefer larger HDL as acceptors [7] (Fig. 32.2, right part). HDL also acquire lipids from chylomicrons and VLDL in the course of hydrolysis of TG by LPL.

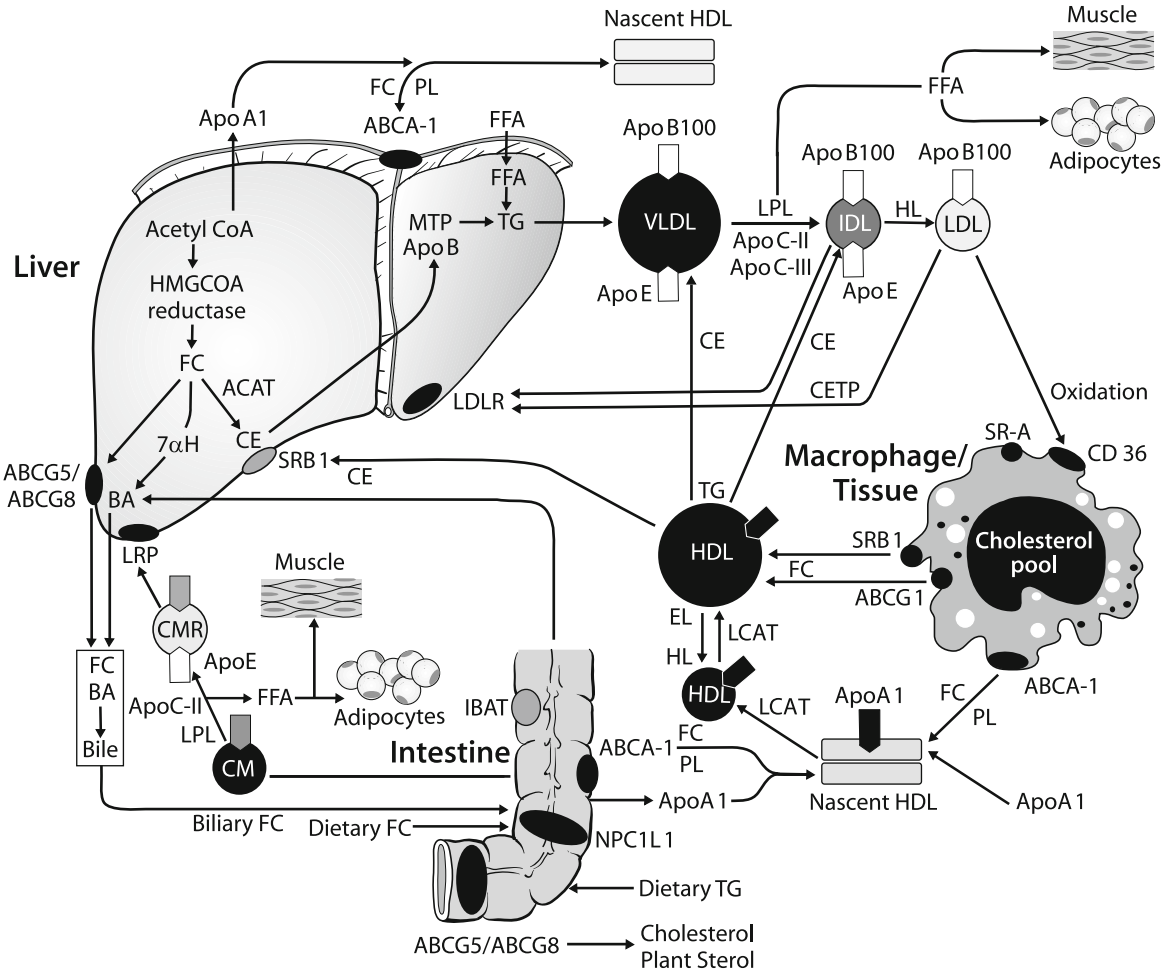


Fig. 32.2. The reverse cholesterol transport pathway and its interaction with the exogenous (intestinal) and endogenous (hepatic) pathways. ApoA-I is synthesised and secreted by intestine and liver, after which it interacts with ATP-binding cassette (ABC) protein A1, promoting the egress of FC and phospholipids (PL), and the formation of the nascent HDL particle. Lecithin cholesterol acyl transferase (*LCAT*) and apoA-I catalyse the formation of CE by adding a FFA from the PL to FC, producing a spherical HDL particle, which becomes larger through LCAT activity. The mature, larger HDL exchanges some of its CE for TG from the apoB-containing lipoproteins, which are then removed by the low density lipoprotein receptor. The CE on large HDL can also be delivered to the liver by specific uptake of the scavenger receptor class B type I (SR-BI) receptor. Once inside the liver, cholesterol must be excreted into bile directly through ABCG5/ABCG8 or converted into BA by 7αH to complete the reverse cholesterol transport pathway. (Reproduced with permission from [63])

Transfer of Lipid Between HDL and the ApoB-containing Lipoproteins. CE are transferred from the core of mature HDL to TG-rich lipoproteins, a reaction promoted by cholesteryl ester transfer protein (CETP) (◘ Table 32.3), which exchanges CE from HDL for TG from TG-rich lipoproteins [8] (◘ Fig. 32.2, central part). This exchange depletes CE but enriches TG in HDL [7]. Phospholipid transfer protein (PLTP) (◘ Table 32.3) is structurally similar to CETP and mediates the transfer of unsaturated fatty acids on PL from the apoB-containing lipoproteins to HDL, contributing to the acquisition of PL by HDL.

Reverse Cholesterol Transport. CE on spherical HDL can be transported back to the liver by two mechanisms. CE are transferred from HDL by CETP to the apoB-containing lipoproteins, which are then cleared by the LDLR. CE may also be delivered directly to the liver through SR-BI, also commonly called the 'HDL receptor' [9], although SR-B1 can bind other major lipoproteins (see also below). Once CE are delivered to the liver and hydrolysed into FC and FFA, the FC can be excreted directly into the bile, or converted into bile acids by 27-α-hydroxylase. Both these pathways result in delivery of sterol from peripheral tissues through plasma into hepatocytes, promoting the excretion of sterols into the stool. Reverse cholesterol transport is postulated to explain, at least in part, the protective effect that HDL and apoA-I have against the development of atherosclerosis. Conversely, factors that impede this process, such as a dysfunctional HDL, appear to promote atherosclerosis.

In addition to their participation in reverse cholesterol transport, HDL may be cardioprotective through their antioxidant, anti-inflammatory and antithrombotic effects [7, 8]. HDL impedes LDL oxidation by metal ions, an effect that may be due to the influence of several molecules on HDL, including apoA-I, platelet-activating factor acetylhydrolase and paraoxonase [7, 8]. Accumulation of large HDL2 (◘ Table 32.1), thought to be the most cardioprotective of the HDL subclasses, is favoured by oestrogens, which negatively regulate HL. In contrast, progesterone and androgens, which positively regulate HL, lead to increased production of small HDL3 (◘ Fig. 32.2).

Lipid-altering Drugs: Pharmacological Manipulation of the Metabolic and Cellular Processes of Lipid and Lipoprotein Metabolism. The major biochemical mechanisms of the effect of the six major classes of drugs on lipoprotein metabolism is schematically depicted in ◘ Fig. 32.1. Their clinical implications are discussed in the ► 'Treatment' section below.

32.2 Disorders of Exogenous Lipoprotein Metabolism

The two classic disorders of exogenous lipoprotein metabolism are defective or missing LPL and defective apoC-II, the co-factor for LPL (◘ Fig. 32.1). Both involve decreased removal of chylomicrons. Several more recently reported disorders involving chylomicron catabolism include: homozygous loss of function mutations in *APOA5;* deficiency in glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1); and frameshift mutations in the cell surface protein caveolin-1 [1].

32.2.1 Lipoprotein Lipase Deficiency

Familial LPL deficiency is a rare, autosomal recessive condition that affects about 1 in 1 million children and results from a variety of mutations in the *LPL* gene [1]. The parents of affected children are often consanguineous. Patients with classic LPL deficiency present in the first months of life with striking hypertriglyceridaemia, often ranging between 5,000 to 10,000 mg/dl with a total cholesterol (TC) between 500 and 1,000 mg/dl (◘ Table 32.4). This disorder is often suspected because of colic, creamy plasma on the top of a haematocrit tube, hepatosplenomegaly or eruptive xanthomas. Usually only the chylomicrons are elevated (type I phenotype) (◘ Table 32.5), but occasionally both chylomicrons and VLDL are elevated (type V phenotype). Findings later in childhood include lipaemia retinalis, abdominal pain and pancreatitis, a life-threatening complication of the massive elevation in chylomicrons. Premature atherosclerosis is unusual because the CM are too large to enter the vascular wall.

When chylomicrons are markedly increased they can replace water (volume) in plasma, producing artefactual decreases in concentrations of plasma constituents; for example, for each 1,000-mg/dl increase in plasma TG, serum sodium levels decrease by between 2 and 4 meq/l.

The diagnosis is first made by a test for post-heparin lipolytic activity (PHLA). The mass of LPL released can also be assessed, using an ELISA assay. Parents of LPL-deficient patients often have LPL activity halfway between those of normal controls and their LPL-deficient child. The parents may be moderately hypertriglyceridaemic or have normal levels.

Treatment is a diet very low in fat (10-15% of calories) [10]. Lipid-lowering medication is ineffective. Affected infants can be given a special formula enriched in medium-chain TG (MCT), which do not require the formation of chylomicrons for absorption. A subset of LPL-deficient

Table 32.4. Guidelines for plasma triglyceride levels in adults

Triglyceride levels		Category
mg/dl	mmol/l	
<150	<1.71	Desirable
150-199	1.71-2.67	Borderline
200-399	2.28-4.55	Elevated
400-999	4.56-11.39	High
>1,000	>11.40	Very high

Table 32.5. Lipoprotein phenotypes of hyperlipidaemia

Lipoprotein phenotype	Elevated lipoprotein
Type I	Chylomicrons
Type IIa	LDL
Type IIb	LDL, VLDL
Type III	Cholesterol-enriched IDL
Type IV	VLDL
Type V	Chylomicrons, VLDL

patients with unique, possibly posttranscriptional, genetic defects respond to therapy with MCT oil or omega-3 fatty acids by normalising fasting plasma TG; a therapeutic trial with MCT oil should, therefore, be considered in all patients [10]. MCT oil can improve the palatability and caloric content of a very low-fat diet. Affected infants and children must get at least 1% of their calories from the essential fatty acid, linoleic acid.

32.2.2 Apo C-II Deficiency

Marked hypertriglyceridaemia (TG >1,000 mg/dl) can also present in patients with a rare autosomal recessive disorder affecting apoC-II, the co-factor for LPL. Affected homozygotes have very high TG ranging from 500 to 10,000 mg/dl (Table 32.4). The disorder is often delayed into adulthood and is suspected by creamy plasma or recurrent bouts of pancreatitis. A type V lipoprotein phenotype (Table 32.5) is typical. Eruptive xanthomas and lipaemia retinalis may be present, but those with apoC-II deficiency usually do not get premature atherosclerosis.

The PHLA test shows very low LPL activity. Addition of normal plasma with apoC-II to the PHLA test restores normal LPL activity. The apoC-II level is very low to undetectable, using an ELISA assay. Apo C-II deficiency is caused by a variety of mutations. Obligate heterozygous carriers of apo C-II mutants usually have normal plasma lipid levels, despite a 50% reduction in apoC-II.

The treatment of patients with apoC-II deficiency also requires a very low fat diet. Infusion of normal plasma in vivo to an affected patient decreases TG.

32.3 Disorders of Endogenous Lipoprotein Metabolism

These disorders are quite heterogeneous. For example, in some patients there is increased synthesis of TG, but apoB production is not proportionally increased (familial hypertriglyceridaemia [FHT]), while in others there is increased production of both TG and apoB, resulting in increased VLDL, IDL and LDL (familial combined hyperlipidaemia [FCHL]).

32.3.1 Disorders of VLDL Overproduction

- **Familial Hypertriglyceridaemia**

Patients with FHT most often present with elevated levels of TG but normal LDL-C levels (type IV lipoprotein phenotype) (Table 32.5). FHT has been associated with mutations in the apolipoprotein A5 gene (*APOA5*) (Table 32.2) and the lipase I gene (*LIPI*), and with polymorphism in the *RP1* gene [1].

FCHL may be autosomal dominant with reduced penetrance in children. The diagnosis is confirmed by finding at least one first-degree relative with a similar type IV lipoprotein phenotype. VLDL levels may increase to a considerable degree, leading to hypercholesterolaemia as well as marked hypertriglyceridaemia (>1,000 mg/dl) and occasionally to hyperchylomicronaemia (type V lipoprotein phenotype) (Table 32.5). This extreme presentation of FHT is usually due to the effects of obesity and type 2 diabetes mellitus. LDL-C and apoB levels remain normal, or low normal, despite levels of TG that can exceed 500 mg/dl.

Patients with FHT often manifest hyperuricaemia, glucose intolerance, obesity and peripheral vascular disease, but a family history of premature CAD may not be present. Pancreatitis may occur in the rare patient with a type V lipoprotein phenotype. The metabolic defect in FHT appears to be due to the increased hepatic production of TG, but the production of apo B-100 is not increased. This results in the enhanced secretion of very large VLDL particles that are not hydrolysed at a normal rate by LPL and apoC-II.

Diet, and particularly a reduction to ideal body weight, is the cornerstone of therapy in FHT. For patients with persistent hypertriglyceridaemia above 400 mg/dl, treatment with fibric acid derivatives, niacin or omega-3 fish oils may reduce the elevated TG by up to 50%.

■ Familial Combined Hyperlipidaemia and the Small Dense LDL Syndromes

■■ Clinical Presentation

Patients with FCHL may present with elevated LDL-C alone (type IIa lipoprotein phenotype), normal LDL-C but elevated TG (type IV lipoprotein phenotype), or with both LDL-C and TG elevated (type IIb lipoprotein phenotype) (◘ Table 32.5). The diagnosis of FCHL is confirmed by the finding of one, or preferably more, first-degree family members who have a different lipoprotein phenotype from the proband. Other characteristics of FCHL include the presence of an increased number of *small, dense LDL particles,* which link FCHL to other disorders, including hyperapobetalipoproteinaemia (hyper apoB), LDL subclass pattern B and familial dyslipidaemic hypertension [11]. In addition to hypertension, patients with FCHL and the small-dense LDL syndromes can also manifest hyperinsulinism, glucose intolerance, low HDL-C levels and increased visceral obesity (syndrome X or metabolic syndrome).

From a clinical prospective, FCHL and other small, dense LDL syndromes clearly aggregate in families with premature CAD, and as a group, these disorders are the most commonly recognised dyslipidaemias associated with premature CAD; they may account for one third, or more, of families with early CAD.

■■ Metabolic Derangement

There are three metabolic defects that have been described both in FCHL and hyperapoB patients: (1) overproduction of VLDL and apoB-100 in liver; (2) slower removal of chylomicrons and chylomicron remnants; and, (3) abnormally increased FFA levels [11, 12].

The abnormal FFA metabolism in FCHL and hyperapoB subjects may reflect the primary defect in these patients. Impaired metabolism of postprandial intestinally derived TG-rich lipoproteins is present. Decreased inhibition of hormone-sensitive lipase by insulin in adipocytes leads to increased plasma FFA. Fatty acids and glucose compete as oxidative fuel sources in muscle, such that increased concentrations of FFA inhibit glucose uptake in muscle and result in insulin resistance. Finally, elevated FFA may drive hepatic overproduction of TG and apo B.

A cellular defect in the adipocytes of hyperapoB patients prevents the normal stimulation of FFA incorporation into TG by a small-molecular-weight basic protein called the acylation-stimulatory protein (ASP) [13]. The active component in chylomicrons responsible for enhancement of ASP in human adipocytes appears to be transthyretin, a protein that binds retinol-binding protein and complexes thyroxin and retinol [13, 14]. The peroxisome proliferator activator receptors, which are retinoic acid dependent, may also be affected. A defect in a basic protein receptor involved in phosphorylation and signal transduction appears to be present in a subset of patients with hyperapoB and premature CAD [15].

■■ Genetics

The basic genetic defect(s) in FCHL and the other small, dense LDL syndromes are not known. FCHL and these other syndromes are clearly genetically heterogeneous, and a number of genes (oligogenic effect) may influence the expression of FCHL and the small dense LDL syndromes [11, 16].

■■ Treatment and Prognosis

The treatment of FCHL and hyperapoB starts with a diet reduced in total fat, saturated fat, *trans* fat and cholesterol. This reduces the burden of postprandial chylomicrons and chylomicron remnants (which appear to be atherogenic). Reduction to ideal body weight may improve insulin sensitivity and decrease VLDL overproduction. Regular aerobic exercise is important. Two classes of drugs, fibrates and niacin (nicotinic acid), lower TG and increase HDL-C and also convert small, dense LDL particles to normal-sized LDL particles. The HMG-CoA reductase inhibitors (statins) are very effective in lowering LDL-C and the total number of atherogenic LDL particles, but their effects on HDL-C and TG are of smaller magnitude. In many patients with FCHL, combination therapy of a statin with either a fibrate or niacin will be required to obtain the most optimal lipoprotein profile [9] (▶ Section 32.7).

■ Lysosomal Acid Lipase Deficiency: Wolman Disease and Cholesteryl Ester Storage Disease (CESD)

■■ Clinical Presentation

Historically, Wolman disease is a fatal disorder with a life- span of generally under 1 year [17]. Clinical manifestations start in the first weeks of life with persistent and forceful vomiting, associated with marked abdominal distension, watery stools, severe anaemia and failure to thrive. Hepatosplenomegaly is invariably present and may be of massive proportion. The most striking feature is calcification of the adrenal glands. Circulating vacuolated lymphocytes and foam cells in bone marrow are almost constant findings.

In contrast to Wolman disease, CESD is characterised by a relatively variable and mild phenotype [18]. The prin-

cipal and sometimes only sign, hepatomegaly, may be evident at birth or in early childhood, increases with time, and eventually leads to hepatic fibrosis. Recurrent abdominal pain is frequent. Acute or chronic liver failure and jaundice have been observed. Patients with CESD can survive for longer periods of time [18]. In some cases, patients with CESD have developed premature atherosclerosis.

■■ Metabolic Derangement

Both Wolman disease and CESD are caused by lysosomal acid lipase (LAL) deficiency. LAL is an important lysosomal enzyme that hydrolyses LDL-derived CE to FC. Intracellular levels of FC are important in regulating cholesterol synthesis and LDLR activity (see also above). In LAL deficiency, CE are not hydrolysed in lysosomes and do not generate FC. In response to low levels of intracellular FC, cells continue to synthesise cholesterol and apo B-containing lipoproteins.

Wolman disease and CESD are autosomal recessive disorders attributable to mutations in the LAL gene on chromosome 10. Diagnosis relies on leukocyte lipase and molecular investigations.

■■ Treatment

Lovastatin reduces both the rate of cholesterol synthesis and the secretion of apo B-containing lipoproteins, leading to significant reductions in TC, LDL-C and TG in CESD [18]. Infants with Wolman's disease respond either to transplantation of unrelated HLA-mismatched umbilical cord-blood derived stem cells, which restore normal LAL activity before permanent end-organ damage [19] or to haematopoietic cell transplantation [20].

32.3.2 Disorders of LDL Removal

These disorders, characterised by marked elevations of TC and LDL-C, provided the initial insights into the role of LDL in human atherosclerosis. Six monogenic diseases that cause marked hypercholesterolaemia are discussed: familial hypercholesterolaemia (FH); familial ligand-defective apo B-100 (FDB); heterozygous FH3; autosomal recessive hypercholesterolaemia (ARH); sitosterolaemia; and cholesterol 7-α-hydroxylase deficiency. While their underlying molecular defects may differ, the end result is to reduce LDLR activity and raise LDL-C.

■ Familial Hypercholesterolaemia (LDLR Defect)

■■ Clinical Presentation

FH is an autosomal codominant disorder that presents in heterozygotes as a 2- to 3-fold elevation in TC and LDL-C levels [5]. FH is completely expressed at birth and early in childhood [21]. FH is strongly associated with premature CAD, and by age 50 about half of untreated heterozygous FH males and 25% of affected females will develop CAD. Heterozygotes develop tendon xanthomas in adulthood, often in the Achilles tendons and the extensor tendons of the hands. FH homozygotes often have cholesterol levels between 600 and 1,000 mg/dl and usually develop CAD and life-threatening supravalvular aortic stenosis in their 2nd decade. Most FH homozygotes have planar xanthomas by the age of 5 years, notably in the webbing of fingers and toes, the knees and the buttocks.

■■ Metabolic Derangement and Genetics

FH is one of the most common inborn errors of metabolism and affects 1 in 500 worldwide [5] (◘ Table 32.6). FH has a higher incidence in certain populations, such as Afrikaners, Christian Lebanese, Finns and French-Canadians, which is due to founder effects. FH is due to one of more than 900 different mutations in the *LDLR* gene [5]. About 1 in 1 million children inherit two mutant alleles in *LDLR*, presenting with a 4- to 8-fold increase in LDL-C levels. Most FH homozygotes inherit two different mutant alleles in *LDLR* (genetic compounds), but some have two identical *LDLR* mutations (true homozygotes). Mutant alleles may: fail to produce LDLR proteins (null alleles); encode receptors blocked in intracellular transport between ER and Golgi (transport-defective alleles); produce proteins that cannot bind LDL normally (binding defect); bind LDL normally, but not internalise LDL (internalisation defects); and disrupt the normal recycling of the LDLR back to the cell surface (recycling defects) [5].

Prenatal diagnosis of FH homozygotes can be performed by assays of LDLR activity in cultured amniotic fluid cells, direct DNA analysis of the molecular defect(s) or linkage analysis using tetranucleotide DNA polymorphisms.

■■ Treatment

The dietary treatment of FH can be supplemented with plant sterols or stanols to decrease cholesterol absorption. Most FH heterozygotes require higher doses of more potent statins to lower LDL-C sufficiently. The addition of a bile acid-binding sequestrant (BAS) or a cholesterol absorption inhibitor (CAI) to a statin is often necessary to lower LDL-C to target levels. Decreased LDLR activity can also lead to borderline hypertriglyceridaemia and low HDL-C. Niacin (nicotinic acid) can therefore be a very useful adjunct to treatment, increasing HDL-C and lowering TG, and also decreasing elevated Lp (a) lipoprotein. FH homozygotes can respond somewhat to high doses of statins and nicotinic acid, both of which decrease produc-

Table 32.6. Major monogenic diseases that cause decreased LDLR activity and marked hypercholesterolaemia. (Modified with permission from [5])

Disease	Defective gene	Prevalence	LDL-C	Metabolic defect
Autosomal dominant				
FH Heterozygous FH Homozygous FH	LDLR	 1 in 500 1 in 1×10^6	 3X 5X	Decreased LDL clearance (primary) Increased LDL production (secondary)
FDB Heterozygous FDB Homozygous FDB	APOB	 1 in 1,000 1 in 4×10^6	 2X 3X	Decreased LDL clearance
FH3 Heterozygous FH3	PCSK9	 <1 in 2,500	 3X	Unknown
Autosomal recessive				
ARH Sitosterolaemia	ARH ABCG5 or ABCG8	<1 in 5×10^6 <1 in 5×10^6	4X 1X to 5X	Decreased LDL clearance Decreased cholesterol excretion(1^0) Decreased LDL clearance (2^0)

ARH, autosomal recessive hypercholesterolaemia; *FDB*, familial ligand-defective apoB-100; *FH*, familial hypercholesterolaemia; *PCSK9* proprotein convertase subtilisin-like kexin type 9; *ABCG5* and *ABCG8*, ATP-binding cassette half-transporters G5 or G8; *X*, mean LDL-cholesterol (*LDL-C*) level in normals

tion of hepatic VLDL, leading to decreased production of LDL. A CAI inhibitor also lowers LDL by about 25% in FH homozygotes. In the end, however, FH homozygotes inevitably require weekly LDL apheresis to lower LDL-C into a less atherogenic range. If LDL apheresis cannot be performed, then hepatic transplantation may be considered. In the future, ex vivo gene therapy for FH homozygotes may become the treatment of choice.

Phenocopies of FH Homozygotes. The other four primary disorders affecting LDLR activity (Table 32.6) can also be present in affected children and adolescents with planar, tendon, or tuberous xanthomas and in adolescents with the dominant form of dysbetalipoproteinaemia (see below). Patients with secondary disorders of dyslipidaemia accompanied by xanthomas include biliary cirrhosis, congenital biliary atresia, Alagille syndrome, myelomas and Wolman disease [5]. These disorders have other clinically salient findings to distinguish them from FH homozygotes.

▪ Familial Ligand-defective ApoB

Heterozygotes with familial ligand-defective apoB (FDB) may present with normal, moderately elevated, or markedly increased LDL-C levels [5] (Table 32.6). Hypercholesterolaemia is usually not as extreme in FDB as in patients with heterozygous FH. About 1 in 20 of affected patients with FDB have tendon xanthomas and more extreme hypercholesterolaemia. This disorder represents a small fraction of patients with premature CAD, i.e. no more than 1%.

In FDB patients, there is delayed removal of LDL from blood despite normal LDLR activity, but the clearance of VLDL remnants and IDL TG-enriched particles is not affected.

The most commonly recognised mutation in FDB is a missense mutation (R3500Q) in the LDLR-binding domain of apoB-100 [21]. The frequency of FDB heterozygotes is about 1 in 1,000 in central Europe [5], but appears less common in other populations (Table 32.6).

Dietary and drug treatment of FDB is similar to that used in FH heterozygotes.

▪ Heterozygous FH3

The clinical phenotype of heterozygous FH3 is indistinguishable from that seen in FH heterozygotes [5, 22]. FH3 does not segregate with *LDLR*, and results from mutations in proprotein convertase subtilisin-like kexin type 9 (PCSK9) [5, 22]. PCSK9 is a serine protease that facilitates the degradation of LDLR [22], but the exact mechanism(s) is not known. Gain-of-function mutations that increase PCSK9 activity decrease LDLR activity, producing a phenotype similar to that of FH. Conversely, loss-of-function mutations that decrease PCSK9 activity increase LDLR and cause levels of LDL-C <80 mg/dl. Drugs that inhibit PCSK9 activity are being developed and are promising for lowering LDL-C, either alone or in combination with a statin.

▪ Autosomal Recessive Hypercholesterolaemia (ARH)

ARH is a rare autosomal recessive disorder that usually presents with LDL-C levels between those found in FH heterozygotes and FH homozygotes [5]. ARH patients often have large tuberous xanthomas. Their onset of CAD is often later than that of FH homozygotes. To date, most of the families reported have been Sardinian or Lebanese. The LDL-C levels in the parents are usually normal, but can be elevated. Strikingly, in ARH there is normal LDLR activity in fibroblasts, but this is defective in lymphocytes. To date at least ten mutations have been described in the *ARH* gene [5].

Fortunately, patients with ARH respond quite dramatically to treatment with statins, but some will also require LDL apheresis. A BAS or CAI may be added to the statin to effect a further reduction in LDL-C.

▪ Sitosterolaemia

Patients with this rare, autosomal recessive condition can present with normal to markedly elevated TC and LDL-C levels, tendon and tuberous xanthomas and premature CAD [5]. Homozygotes manifest abnormal intestinal hyperabsorption of plant (sitosterol, campesterol, and stigmasterol) and shellfish sterols, and of cholesterol. In normal humans, very small quantities of plant sterols are absorbed and plasma plant sterol levels are low (0.3 to 1.7 mg/dl), constituting <1% of plasma total sterol. The levels of total plant sterols (13-37 mg/dl) in sitosterolemics are very elevated and represent 7-16% of the total plasma sterols. Patients often present in childhood with striking tuberous and tendon xanthomas, despite normal or FH heterozygote-like LDL-C levels. The clinical diagnosis is made by documenting elevated plant sterols using gas-liquid chromatography. The parents usually have normal TC and plant sterol levels.

Two ABC half-transporters, ABCG5 and ABCG8 [5], together normally limit the intestinal absorption of plant sterols and cholesterol and promote their excretion. Sitosterolaemia is caused by two mutations in either of the two adjacent genes that encode ABCG5 or ABCG8 (◘ Table 32.6), thereby enhancing absorption of dietary sterols. This leads to an increased hepatic content of cholesterol and plant sterols, suppression of *LDLR,* inhibition of LDLR synthesis and elevated LDL-C levels.

Dietary treatment is paramount in sitosterolaemia and consists of a diet very low in both cholesterol and plant sterols. Thus, in contrast to the standard low-cholesterol, low-saturated-fat diet, plant foods with high-fat, high-plant-sterol (e.g. oils and margarines) content must be avoided. BAS are particularly effective in lowering plant and LDL sterol levels. A CAI, ezetimibe, is also quite effective [23]. These patients have a poor response to statins.

▪ Cholesterol 7α-Hydroxylase Deficiency

A few patients have been described with a deficiency in the rate-limiting enzyme of bile acid synthesis, cholesterol 7α-hydroxylase, which converts cholesterol into 7α-hydroxy-cholesterol (▶ Chapter 34, ◘ Fig. 3.1). Both hypercholesterolaemia and hypertriglyceridaemia have been reported [24]. As with patients with sitosterolaemia, these subjects were relatively resistant to statin therapy [24].

32.4 Disorders of Endogenous and Exogenous Lipoprotein Transport

32.4.1 Dysbetalipoproteinaemia (Type III Hyperlipoproteinaemia)

Adults with dysbetalipoproteinaemia present with elevations in both TC and TG, usually but not always above 300 mg/dl. The hallmark of the disorder is the presence of VLDL that migrate as beta lipoproteins (β-VLDL) rather than prebeta lipoproteins (type III lipoprotein phenotype) (◘ Table 32.5). β-VLDL reflects the accumulation of cholesterol-enriched remnants of both hepatic VLDL and intestinal chylomicrons (◘ Fig. 32.1) [25]. These remnants result from the presence of a dysfunctional apoE, the ligand for receptor-mediated removal of both chylomicron and VLDL remnants by the liver.

Premature atherosclerosis of the coronary, cerebral and peripheral arteries is often present. Xanthomas are common, especially planar lesions in the creases of the palms and tuberoeruptive xanthomas over the knees or buttocks. Occasionally, tuberous and tendon xanthomas are found. Hyperuricaemia and glucose intolerance occur in up to half the patients with this syndrome.

Human apoE exists as three major isoforms (E2, E3 and E4), each of which is specified by an independent allele at the locus for the *apoE* gene [25]. One in 100 persons is homozygous for the *apoE2* allele, which results in decreased affinity of the TG-enriched remnants to their hepatic receptors; however, because the prevalence of this disorder is only 1:10,000, other modifying factors, such as hypothyroidism, low-oestrogen state, obesity or diabetes are necessary for full-blown clinical expression. This recessive form of dysbetalipoproteinaemia has a delayed penetrance beyond childhood.

A dominant form of dysbetalipoproteinaemia is caused by the expression of one of several rare variants of apoE that usually involve the substitution of neutral or acidic amino acids for basic ones in the region of apoE that interacts directly with the LDLR [25]. The dominant form can be expressed in childhood and does not require the presence of modifying factors.

The diagnosis of dysbetalipoproteinaemia is based on: (1) demonstration of E2E2 genotype; (2) the presence of β-VLDL; and (3) a cholesterol-enriched VLDL (VLDL-C/TG ratio >0.30). LDL and HDL-C levels are low or normal.

Patients with this disorder are highly responsive to a low-fat diet and drug treatment with a fibric acid derivative, although nicotinic acid and statins may also be effective.

32.4.2 Hepatic Lipase Deficiency

Patients with HL deficiency can present with features similar to those of dyslipoproteinaemia (type III hyperlipoproteinaemia), including hypercholesterolaemia, hypertriglyceridaemia, accumulation of TG-rich remnants (including β-VLDL), planar xanthomas and premature cardiovascular disease [26]. Recurrent bouts of pancreatitis have been described.

HL shares a high degree of homology to LPL and pancreatic lipase. HL hydrolyses both TG and PL in plasma lipoproteins and converts IDL to LDL and large HDL_2 to HDL_3. In HL deficiency, therefore LDL-C is usually low and HDL-C is often quite high (despite the hypertriglyceridaemia) (◘ Fig. 32.1, Fig. 32.2).

HL deficiency is rare and is inherited as an autosomal recessive trait. Obligate heterozygotes are normal. The diagnosis is made by a PHLA test to determine that HL activity is absent but LPL activity is normal.

Treatment includes a low total-fat diet. In one report, the hypercholesterolaemia and hypertriglyceridaemia in HL deficiency improved dramatically on treatment with lovastatin, while gemfibrozil reduced TG but elevated cholesterol [26].

32.5 Disorders of Reduced LDL-Cholesterol Levels

32.5.1 Abetalipoproteinaemia

Abetalipoproteinaemia is a rare, autosomal recessive disorder in patients with undetectable plasma apoB levels [27]. Patients present with symptoms of fat malabsorption and neurological problems. Fat malabsorption occurs in infancy, with symptoms of failure to thrive (poor weight gain and steatorrhoea) and lipid vacuoles invading enterocytes visible on intestinal biopsy. Fat malabsorption is due to the inability to assemble and secrete chylomicrons from enterocytes. Neurological problems begin during adolescence and include: dysmetria; cerebellar ataxia; spastic gait; and axonal peripheral neuropathy mimicking vitamin E malabsorption or Friedreich ataxia. Other manifestations include atypical retinitis pigmentosa, anaemia, arrhythmias and myopathy.

TC levels are exceedingly low (20-50 mg/dl), and no detectable levels of the apoB-containing lipoproteins, i.e. chylomicrons, VLDL, or LDL are present. HDL levels are measurable but low. Vitamin E levels are extremely low. Acanthocytes are seen on a blood film. Parents have normal lipid levels.

The absence of plasma apoB levels was first believed to be due to defects in the *APOB* gene, but the defect in synthesis and secretion of apoB was secondary to the absence of MTP that normally permits the transfer of lipid to both apoB-48 and apoB-100 [27]. MTP is a heterodimer composed of the ubiquitous multifunctional protein, protein disulfide isomerase, and a unique 97-kDa subunit. Mutations that lead to the absence of a functional 97-kDa subunit cause abetalipoproteinaemia.

Treatment of abetalipoproteinaemia begins with reducing the intake of fat to 5-20 g/day to control steatorrhoea, resulting in marked clinical improvement and growth acceleration. The diet should also be supplemented with linoleic acid (e.g., 5 g corn oil or safflower oil/day). MCT as a caloric substitute for long-chain fatty acids may produce hepatic fibrosis, and thus MCT should be used with caution, if at all. Fat-soluble vitamins should be added to the diet. High-dose oral vitamin E (150-200 IU/kg/day) is essential to prevent or ameliorate neurological and retinal complications. On treatment, vitamin E levels increase but remain low. Adipose tissue may also be used to assess the delivery of vitamin E. Rickets can be prevented by normal quantities of vitamin D, but high doses of vitamin A (200-400 IU/kg/day) may be required to raise the level of vitamin A in plasma to normal. Enough vitamin K (5-10 mg/day) should be given to maintain a normal prothrombin time.

32.5.2 Hypobetalipoproteinaemia

The phenotype of hypobetalipoproteinaemia (hypobeta) is characterised by very low levels of LDL-C, usually defined as the lower fifth percentile of a normal distribution. Plasma TC is low; VLDL-C and TG are low or normal. Hypobetalipoproteinaemia can be primary or secondary to anaemia, dysproteinaemias, hyperthyroidism, intestinal lymphangiectasia with malabsorption, myocardial infarction, severe infections and trauma.

Familial Hypobeta. Familial hypobeta is inherited in an autosomal dominant manner and may or may not be as-

sociated with mutations in *APOB.* Affected individuals are usually asymptomatic; the prevalence of CVD is low; and many patients have long lives. Those with a defect in *APOB* can have about 3-fold the normal amount of hepatic fat. A relatively large number of mutations in the *APOB* gene causing hypobeta have been described [28]. Almost all of them are either nonsense or frameshift mutations that create a premature stop codon and a truncated apoB-100. Familial hypobeta has also been linked to a susceptibility locus on chromosome 3p21, and in some families is linked neither to *APOB* nor to chromosome 3p21 [28].

Loss-of-function Mutations in *PCSK9*. The phenotype of hypobeta is also found in those with a loss-of-function mutation in the *PCSK9* gene [22]. In this case, the low LDL results not from decreased production of LDL but from enhanced LDLR activity due to the decreased PCSK9 function [22] (see also above). Patients with this cause of familial hypobetalipoproteinaemia also have a considerable lifelong reduction in CVD [29].

- **Homozygous Hypobetalipoproteinaemia**

The clinical presentation of children with this disorder depends on whether they are homozygous for null alleles in *APOB* (i.e. make no detectable apoB) or homozygous (or compound heterozygotes) for other alleles, which produce lipoproteins containing small amounts of apoB or a truncated apoB [30]. Null-allele homozygotes are similar phenotypically to those with abetalipoproteinaemia and may have fat malabsorption, neurological disease and haematological abnormalities as their prominent clinical presentation, and require similar treatment. However, the parents of these children are heterozygous for hypobetalipoproteinaemia, in contrast to parents of abetalipoproteinaemic children, who are normolipidaemic.

32.5.3 Chylomicron Retention Disease

Chylomicron retention disease (CRD), or Anderson's disease, is another rare genetic disease that causes malnutrition, failure to thrive, growth failure, vitamin E deficiency and other complications [31, 32] The diagnosis is based on a history of chronic diarrhoea with fat malabsorption and much decreased but not absent LDL-C and apoB; in contrast to abetalipoproteinaemia and homozygous hypobetalipoproteinaemia, the TG are normal in CRD [32]. Fat-laden enterocytes and vitamin E deficiency are invariably present. Hepatic steatosis is common. Increased creatine kinase levels and cardiomyopathy may reflect the muscular complications. Neurological and ophthalmological complications in CRD are less severe than in other types of familial hypocholesterolaemia. The molecular defects in CRD or Anderson's disease are due to mutations in *SAR1B,* leading to a defective Sar1b protein that prevents the normal transport of prechylomicrons from the ER to the Golgi apparatus [32]. Vomiting, diarrhoea and abdominal distension improve on a low-long-chain fat diet made up of uniquely polyunsaturated fatty acids. Essential fatty acid deficiency is especially severe early in life. Besides diet, treatment includes fat-soluble vitamin supplements, especially large amounts of vitamin E to prevent neurological complications.

32.6 Disorders of Reverse Cholesterol Transport

32.6.1 Familial Hypoalphalipoproteinaemia

Hypoalphalipoproteinaemia is a relatively uncommon phenotype defined as a low level of HDL-C (<5th percentile, age and sex specific) in the presence of normal lipid levels [33, 34]. Patients with this syndrome can have a significantly increased prevalence of CAD, but do not manifest the clinical findings typical of other forms of HDL deficiency. Low HDL-C levels of this degree are most often secondary to disorders of endogenous TG metabolism.

In some families, hypoalphalipoproteinaemia behaves as an autosomal dominant trait, but the basic defect is usually unknown. It is likely that the aetiology of primary low HDL-C levels is oligogenic, i.e. that there are significant effects of several genes being expressed [34].

32.6.2 Apolipoprotein A-I Mutations

APOA1 exists on chromosome 11 as part of a gene cluster with *APOC3* and *APOA.4.* A variety of molecular defects have been described in *APOA1*, including gene inversions, gene deletions, and nonsense and missense mutations [33]. Homozygous gene deletions or nonsense mutations are rare and exhibit little if any biosynthesis of apoA-I by the liver and intestine (◘ Fig. 32.2). The virtual absence of apoA-I is accompanied by marked decreases in HDL-C. Obligate heterozygotes, as well as the homozygotes, develop premature CVD. In addition to precocious CVD, homozygotes can manifest other clinical findings of peripheral cholesterol deposition, such as retinopathy, cataracts and xanthomas. Missense mutations in *APOA1* have been

described in kindreds with low HDL-C levels. However, the relationship to premature CAD is much less clear.

32.6.3 Tangier Disease

Tangier disease is an autosomal recessive disorder in which HDL-C levels are extremely low and of an abnormal composition (HDL Tangier or T). HDL_T are chylomicron-like particles, which disappear when a patient consumes a low-fat diet [33].

The characteristic clinical findings in Tangier patients include the presence of enlarged orange yellow tonsils, splenomegaly and a relapsing peripheral neuropathy (► Chapter 2). The finding of orange tonsils is due to the deposition of beta carotene-rich CE in foam cells in the lymphatic tissue. Other sites of foam cell deposition include the skin, peripheral nerves, bone marrow, and rectum. Mild hepatomegaly, lymphadenopathy and corneal infiltration (in adulthood) may also occur.

APOA1 is normal in Tangier patients. The underlying defect is a deficiency in ABCA1 [35]. The very low HDL-C is due to the lack of cholesterol efflux by the deficient ABCA1 to nascent HDL, which can be measured in fibroblasts [35]. Some but not all patients with Tangier disease have premature CAD in adulthood [33-35]. Treatment with a low-fat diet diminishes the abnormal lipoprotein species.

32.6.4 Lecithin-cholesterol Acyltransferase Deficiency

Lecithin-cholesterol acyltransferase (LCAT) is an enzyme located on the surface of HDL particles and is important in transferring fatty acids from the sn-2 position of phosphatidylcholine (lecithin) to the 3-β-OH group on cholesterol (■ Table 32.3). In this process, lysolecithin and esterified cholesterol are generated (α-LCAT). Esterification can also occur on VLDL/LDL particles (β-LCAT).

In patients with **classic LCAT deficiency**, both α- and β-LCAT activity are missing [36]. LCAT deficiency is a rare, autosomal-recessively inherited disorder. More than several dozen mutations in *LCAT*, located on chromosome 16, have been described. The diagnosis should be suspected in patients presenting with low HDL-C, corneal opacifications and renal disease (proteinuria, haematuria). Laboratory tests include measurement of the plasma FC-to-TC ratio. Levels above 0.7 are diagnostic for LCAT deficiency.

In **fish eye disease**, only α-LCAT activity is absent. Patients present with corneal opacifications, but do not have renal disease [36]. Variability in clinical manifestations from patients with fish eye disease may be due to differences in the total LCAT activity.

To date, no therapies exist to treat the underlying defects. Patients succumb primarily to renal disease, and atherosclerosis may be accelerated by the underlying nephrosis. Thus, patients with LCAT deficiency, and other lipid metabolic disorders associated with renal disease, should receive aggressive treatment including a low-fat diet. The secondary dyslipidaemia associated with the nephrotic syndrome responds to statin therapy.

32.6.5 Cholesteryl Ester Transfer Protein Deficiency

The role of CETP in atherosclerosis has not been well defined. CETP is up-regulated in peripheral tissues and liver in response to either dietary or endogenous hypercholesterolaemia. Elevated HDL-C due to deficiency of CETP was first described in Japanese families [37]. Several mutations in CETP are known. The prevalence of CAD in CETP deficiency is not straightforward. In the Japanese kindreds increased CAD was primarily observed with HDL-C of 41-60 mg/dl; with HDL-C >60 mg/dl, men with and without CETP mutations had a low CAD prevalence [37]. Thus, genetic CETP deficiency may or may not be an independent risk factor for CAD. These effects occur in spite of lower levels of apoB in CETP deficiency [38].

Owing to its important role in modulating HDL levels, CETP inhibitors have been developed to raise plasma HDL-C. However, many side effects attributed to interference with aldosterone metabolism, including more frequent death from CAD, were found with the first CETP inhibitor (CP529, 414: Torcetrapib) [39]. Other CETP inhibitors (anacetrapib; dalcetrapib), which are thought not to affect aldosterone levels, are still under investigation.

32.6.6 Scavenger Receptor Class B Type I Receptor Deficiency

SR-BI is a functional lipoprotein receptor that participates in the selective uptake of cholesteryl esters (CE) from HDL [40], LDL [41] and VLDL [42] and is regulated by a number of factors .One of its major functions is to mediate the uptake of CE from the core of the lipoprotein. A number of studies have shown significant associations of the *SCARB1* gene single nucleotide polymorphisms (SNPs) and lipid levels [43-45]. Certain SCARB1 variants had significant association with subclinical atherosclerosis as measured by carotid ultrasonography [46].

32.6.7 Deficiency of Endothelial Lipase

Endothelial lipase (EL) is a member of the triglyceride lipase family of proteins that includes LPL and HL. EL is a product of *LIPG* and primarily hydrolyses PL with little TG lipase activity. EL hydrolyses the lipids in HDL more efficiently than all other lipoproteins, converting a larger HDL to a smaller particle (◘ Fig. 32.2). It has been hypothesised that rare loss-of-function EL variants are a cause of high HDL-C [47].

32.6.8 Elevated Lipoprotein(a)

Lipoprotein(a) (Lp[a]) is comprised of the glycoprotein apo(a) covalently bound to apolipoprotein B-100 of LDL through a disulfide bond [48]. Apo(a) is highly homologous to plasminogen but has no protease activity. Lp(a) levels are highly heritable, but the physiological function of Lp(a) is unknown. Plasma Lp(a) levels are almost entirely dependent on the *apo(a)* gene on chromosome 6q27. Elevated Lp(a) levels were considered a risk factor for myocardial infarction and stroke for many years, but evidence for a causal relationship was lacking. Recent studies indicate a causal role for Lp(a) in CVD [49]. Finally, Lp(a) also appears to promote thrombosis by the inhibition by apo(a) of the conversion of plasminogen to plasmin at the surface of endothelial cells [48].

The best method for diagnosis of elevated Lp(a) is the ELISA assay using a monoclonal antibody. The upper limit of normal using this assay is <75 nmol/l.

Niacin and oestrogen can effectively lower Lp(a) levels, while statins and fibrates do not. Although clinical trial evidence is lacking regarding any beneficial effect of specifically lowering Lp(a) on the prevalence of CVD, the general approach taken is to be more aggressive in the treatment of patients with CVD who have elevated Lp(a). If LDL-C is elevated, or even average, a statin can be used to reduce it to <100 mg/dl, at a minimum. Niacin can be added to reduce Lp (a) and to increase HDL-C.

32.7 Guidelines for the Clinical Evaluation and Treatment of Dyslipidaemia

32.7.1 Clinical Evaluation

Clinical evaluation requires a thorough family history, which is reviewed for premature (before 60 years of age) cardiovascular, cerebrovascular and peripheral vascular disease; dyslipidaemia; diabetes mellitus; obesity; and hypertension.

A baseline assessment of dietary intake of total fat, saturated fat, *trans*-fat, cholesterol, simple sugars and calories is best performed by a registered dietitian.

The medical history is focused on the two major complications of dyslipidaemias: atherosclerotic CVD and pancreatitis. The results of previous resting and stress electrocardiograms and coronary arteriography are assessed. Any history of recurrent abdominal pain, fatty food intolerance and pancreatitis is reviewed. The past and current use of lipid-lowering drugs is determined, as well as any history of untoward reactions or side effects. The review of systems includes diseases of the liver, thyroid and kidney, the presence of diabetes mellitus, and operations including transplantation. For women, a menstrual history, including current use of oral contraceptives and postmenopausal oestrogen replacement therapy, is obtained.

The presence of other risk factors for CAD [50, 51] is systematically assessed: cigarette smoking, hypertension, low HDL-C (<40 mg/dl), age (>45 years in men, >55 years in women), diabetes (CAD risk equivalent), obesity, physical inactivity and atherogenic diet. Height and weight are determined to assess obesity using the Quetelet (body mass) index: weight (kg)/height (m^2). An index of 30 or higher is defined as obesity, and one between 25 and 30 is considered overweight. Waist circumference can be measured (abnormal >40 in. in men, >35 in. in women).

Physical examination includes an assessment of tendon, tuberous and planar xanthomas, palpation of the thyroid, assessment of hepatosplenomegaly and deep tendon reflexes (which are decreased in hypothyroidism) and a cardiovascular examination, including checking for bruits in the carotid, abdominal aortic and femoral arteries. The eyes are examined for the presence of xanthelasmas, corneal arcus, corneal clouding, lipaemia retinalis and atherosclerotic changes in the retinal blood vessels.

The clinical chemistry examination includes (at the minimum) a measurement of TC, TG, LDL-C, and HDL-C, a chemistry panel to assess fasting blood sugar, uric acid, tests of liver and kidney function and thyroid-stimulating hormone (TSH). Fasting (10-12 h) is necessary before these tests.

The USA's National Institutes of Health's guideline, Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), also recommends calculating the non-HDL-C level, particularly in individuals with levels of TG >200 mg/dl. Non-HDL-C can be readily calculated by subtracting HDL-C from TC. A fasting specimen is not necessary for this test, since TG and an estimated LDL-C are not being determined. On average, the levels for non-HDL-C should be 30 mg/dl higher than those used for LDL-C (◘ Table 32.9).

An assessment of the number of LDL particles may be important to determine whether there is a disconnect between LDL-C and the particle number. For example, a patient may have a normal LDL-C but an elevated number of LDL particles, indicating that he or she may be at higher risk of CVD than suggested by LDL-C. An apoB measurement (upper limit of normal for adults 120 mg/dl) can be useful in this regard (e.g. patient has elevated apoB but normal LDL-C). ApoB may be measured non-fasting and is well standardised on an international basis. Other assessments of the number of LDL particles can be made using nuclear magnetic resonance (NMR) spectroscopy, density gradient ultracentrifugation and quantitative agarose gel electrophoresis.

Some patients might also have discordance between HDL-C levels and the concentration of the major protein of HDL, apoA-I. Thus, the patient may have a low level of apoA-I in the face of an average HDL-C, or alternatively have normal levels of apoA-I but low HDL-C. The latter scenario could occur in patients using glitazone therapy for the treatment of type 2 diabetes. ApoA-I can also be useful to assess the ratio of apoB to apoA-I, with a value of 1 or higher indicating that the patient is at high risk for CVD.

Given the recent information that Lp(a) is a causal risk factor for CVD, we recommend that all patients undergoing evaluation for dyslipidaemia and/or CVD have baseline Lp(a) measured. The presence of an elevated Lp(a) level indicates that the patient should be treated more aggressively.

Other tests may be ordered when clinically indicated. These include a highly sensitive C-reactive protein (hsCRP), lipoprotein-associated phospholipase A2 (Lp-PLA2) and prothrombotic factors. Homocysteine is no longer measured because treatment of homocysteine does not prevent CVD events. Haemoglobin A1C (HgbA1C) is measured when a patient has known or suspected diabetes mellitus.

32.7.2 Dietary Treatment, Weight Reduction and Exercise

The cornerstone of treatment of dyslipidaemia is a diet reduced in total fat, saturated fat, *trans*-fat and cholesterol [50, 51] (◘ Table 32.7). The addition of 400 IU or more of vitamin E and 500 mg or more of vitamin C is **not** currently recommended as there is no clear evidence that such supplementations decreases risk of CAD, and in fact may impede the treatment of dyslipidaemia [52].

If a patient is obese (Quetelet index >30), or overweight (Quetelet index 25-30), weight reduction will be an important part of the dietary management. This is particularly true if hypertriglyceridaemia or diabetes mellitus are present.

Regular aerobic exercise (goal 1,000 calories per week) is essential in most patients to help control their weight and dyslipidaemia.

◘ **Table 32.7.** National Cholesterol Education Program diets: steps I and II

Step I
- Less than 30% of calories as fat: less than 10% saturated, 10-15% monounsaturated, and up to 10% polyunsaturated
- 55% carbohydrates
- 15-20% protein
- Less than 300 mg cholesterol/day

Step II
- Less than 30% of calories as fat: <7% saturated, 10-15% monounsaturated, and 10% polyunsaturated
- Less than 200 mg cholesterol/day

32.7.3 Goals for Dietary and Hygienic Therapy

Four lipid parameters are used to define abnormal levels and determine therapeutic goals: LDL-C (◘ Table 32.8), TG (◘ Table 32.4), HDL-C (low <40 mg/dl) and non-HDL-C [50]. If the goals for LDL-C are achieved with dietary management alone, drug therapy is not recommended. The recommended goal for TG is a level <150 mg/dl in adults; the ideal goal is <100 mg/dl. Values of TG >200 mg/dl are associated with an increased number of small, dense LDL particles in 80% of patients. A low HDL-C is a value <40 mg/dl. The treatment goal for HDL-C is 45 mg/dl in men and 50 mg/dl in women [50, 51].

The recommendations from the National Cholesterol Education Program (NCEP) [51] offer guidelines for assessing risk and initiating treatment in patients with hypercholesterolaemia. As shown in ◘ Table 32.8, patients with CAD should be placed on a more aggressive diet and simultaneously on lipid-lowering drug therapy.

The value of pharmacologically lowering lipid levels to reduce CVD event rates is well established, but the optimal levels of cholesterol, LDL-C, HDL-C and TG has not yet been determined. As the result of recent clinical trials, the NCEP established updated lipid-lowering guidelines for primary and secondary prevention of CAD [51] (◘ Table 32.8). As before, the threshold of the LDL-C level to initiate drug therapy and the target for treatment

Table 32.8. NCEP-ATP III guidelines for LDL-lowering pharmacotherapy initiation and goals. (Adapted from [50, 51])

Patient category	Initiation of drug therapy LDL-C (mg/dl)	Therapeutic goal LDL-C (mg/dl)
High risk CAD or CAD risk equivalents (10-year risk >20%)	≥100 (<100: consider drug options)[1]	<100 (optional goal: <70)[1]
Moderately high risk No CAD and >2 risk factors (10-year risk 10-20%)[2]	≥130 (100-129: consider drug options)[1]	<130 (optional goal: <100)[1]
Moderate risk No CAD and <2 risk factors (10-year risk ≤20%)	≥160	<130
Lower risk 0–1 risk factor	≥190 (160-189: LDL-lowering drug therapy optional)	<160

CAD, coronary artery disease; *HDL-C*, high-density lipoprotein cholesterol. [1]Drug therapy advisable on the basis of clinical trials. The optional goal of LDL-C in high-risk patients is <70 mg/dl, or in those with high TG (>200 mg/dl), a non-HDL-C <100 mg/dl. The optional goal of LDL-C in moderately risk patients is <100 mg/dl, or in those with high TG, a non-HDL-C <130 mg/dl. [2]Positive risk factors for CAD are cigarette smoking, hypertension, low HDL-C (<40 mg/dl), age (>45 years in men, >55 years in women), diabetes, obesity, physical inactivity and atherogenic diet)

depend on the presence or absence of CAD, CAD risk equivalents, and associated risk factors. The guidelines provide recommendations for complete screening of TC, LDL-C, HDL-C and TG, encouraging the use of plant sterols or stanols, and soluble fibre, and treatment using non-HDL-C (total cholesterol minus HDL-C) guidelines for patients with TG ≥200 mg/dl [50, 51]. For those with hypertriglyceridaemia (>200 mg/dl), the optional targets for the high-risk and moderate-risk groups are a non-HDL-C of <100 mg/dl and <130 mg/dl, respectively.

32.7.4 Low-density-Lipoprotein-lowering Drugs

Agents that will lower LDL-C include *statins, bile acid sequestrants, cholesterol absorption inhibitors* and *niacin* (Table 32.9). The *fibrates* can also modestly reduce LDL-C levels, but in patients with mixed dyslipidaemia LDL-C levels may stay the same or actually increase [53].

The *statins* available in Europe and the U.S.A. include atorvastatin (Lipitor), fluvastatin (Lescol), lovastatin (Mevacor), pravastatin (Pravachol), simvastatin (Zocor) and rosuvastatin (Crestor). The equivalent doses are about: 5 mg rosuvastatin = 10 mg atorvastatin = 20 mg simvastatin = 40 mg lovastatin = 40 mg pravastatin = 80 mg fluvastatin. Atorvastatin and rosuvastatin both have long half-lives of about 17 h. Thus, they can be taken in the morning or evening, in contrast to the other statins, which should be taken at bed time because of their short half-lives.

Statins undergo extensive first-pass metabolism via the hepatic portal system, and typically less than 20% of these agents reaches the systemic circulation [54]. In the liver the statins inhibit the rate-limiting enzyme of cholesterol biosynthesis, HMG-CoA reductase, (Fig. 32.1) The statins also improve endothelial cell function and stabilise unstable plaques [55, 56]. Each of the six classes of statins has been shown to decrease CVD significantly, usually by 30-40%.

Statins are generally well tolerated, and they have an excellent safety profile with minimal side effects. Liver function tests (AST, ALT) should be monitored at baseline, 6-8 weeks after the initiation of treatment and every 4 months for the 1st year. After that, patients on a stable dose of a statin can have their liver function tests monitored every 6 months. Consideration should be given to reducing the dosage of drug, or its discontinuation, should the liver function tests exceed 3 times the upper limits of normal. Between 1/500 and 1/1,000 patients may develop myositis on a statin, which can lead to life-threatening rhabdomyolysis. Rhabdomyolysis is a rare event, occurring at an incidence of 1.2 per 10,000 patient-years [57]. In such a situation *Lipin 1* mutation should be searched for (► Chapter 35). Creatine kinase (CK) should be measured at baseline and repeated if the patient develops muscle aches and cramps. The statin is discontinued if the CK is >5 times the upper limit of normal in those with symptoms of myositis, or >10 times the upper limit of normal in asymptomatic patients.

Three statins, lovastatin, simvastatin and atorvastatin, are metabolised by the CYP3A4 isozyme of the cytochrome P450 microsomal enzyme system and con-

Table 32.9. Effect of drug classes on plasma lipid and lipoprotein levels. (Adapted and modified from [64])

Drug class	TC	LDL-C	HDL-C	TG
Statins	15-60%	20-60%	3-10%	10-30%
Bile acid resins	10-20%	15-20%	3-5%	Variable
Cholesterol absorption inhibitor	10-20%	15-20%	3-5%	5-10%
Niacin	25%	10-15%	15-35%	20-50%
Fibrates Fish oils	15% 15%	Variable Variable	6-15% Variable	20-50% 50%

TC, total cholesterol

sequently have drug interactions with other agents metabolised by CYP3A4. Statins are not safe in pregnant or nursing women, and should not be used in patients with active or chronic hepatic disease or cholestasis because of potential hepatotoxicity.

The bile acid resins or sequestrants of the first (cholestyramine [Questran], colestipol [Colestid]) and second (colesevalam [Welchol]) generations do not enter the bloodstream, but bind bile acids in the intestine [58], preventing their reabsorption through the ileal bile acid transporter (IBAT) (Fig. 32.1). There is a compensatory increase in hepatic cholesterol synthesis that limits the efficacy of the sequestrants. The use of BAS with a statin produces a complementary decrease in LDL-C. The side effects of the first-generation resins include constipation, heartburn, bloating, decreased serum folate levels and interference with the absorption of other drugs [58].

The cholesterol absorption inhibitor ezetimibe inhibits the intestinal absorption of cholesterol derived from diet and from bile by about 50% [58] (Fig. 32.1). The mechanism of action of ezetimibe occurs through the selective inhibition of a newly discovered transporter that moves cholesterol from mixed micelles into the cells of the jejunum [59]. The transporter is a Niemann-Pick C1-like 1 (NPC1L1) protein localised at the brush border of enterocytes. The use of ezetimibe is also associated with a compensatory increase in cholesterol biosynthesis, limiting its efficacy. Ezetimibe can be combined with any of the statin agents, producing, on average, an additional 25% reduction in LDL-C. In the SHARP trial, ezetimibe combined with simvastatin reduced CVD in patients with chronic renal failure without any undue safety concerns. Ezetimibe is usually well tolerated, and there are generally few drug interactions with this drug [58]. Ezetimibe is also available combined with simvastatin in a single formulation (Vytorin). Ezetimibe should not be used for combination therapy with a statin in patients with active liver disease or unexplained persistent elevations in serum transaminases, or those with chronic or severe liver disease. Co-administration of ezetimibe with cholestyramine decreased the levels of ezetimibe, and co-administration with fibrates increased plasma levels of ezetimibe. Ezetimibe should not be used in patients on cyclosporine until more data are available.

Niacin (nicotinic acid) is a water-soluble B-complex vitamin that through its interaction with its receptor GPR109A [60] inhibits the release of FFA from adipose tissue, leading to decreased delivery of FFA to liver and reduced synthesis of TG. Niacin also inhibits the uptake of HDL through its catabolic pathway, prolonging the half-life of HDL and presumably increasing reverse cholesterol transport. Niacin is also the only lipid-altering drug that reduces Lp(a) lipoprotein. In addition, niacin has more recently been found also to have beneficial effects on endothelial function and inflammation [60].

Niacin is commonly prescribed in those patients with the dyslipidaemic triad (low HDL, elevated TG and increased small, dense LDL) (Table 32.9). Niacin is useful in treating FCHL and in those with isolated low HDL-C.

Niacin is associated with a number of side effects. The most common and most annoying is the cutaneous flush that occurs after administration of niacin. Slow-release niacin (Niaspan) is not associated with flushing but has been reported to have a greater propensity to raise the results of liver function tests. A large trial recently designed should provide important information about the benefits and risk of niacin and laropiprant, a prostaglandin receptor antagonist that mitigates niacin-induced flushing, in combination with statin therapy [61].

Niacin should not be used in patients with active peptic ulcer disease or liver disease. Niacin can precipitate the onset of type 2 diabetes mellitus or gout. In patients with borderline or elevated fasting blood sugar or uric acid levels, niacin should be used with care.

32.7.5 Triglyceride-lowering Drugs

Those drugs that can effectively lower TG include niacin, fibrates, omega-3 fish oils and statins (particularly when used at their highest doses; ◘ Fig. 32.1). A 30-50% reduction in TG is often achieved (◘ Table 32.9).

Fibric acid derivatives (fibrates) are agonists for the peroxisome proliferator-activated receptor alpha (PPAR alpha), which up-regulate the gene for LPL and apoA-V and down-regulate the gene for apolipoprotein C-III [62] (◘ Fig. 32.1). Fibrates also up-regulate the gene for apoA-I, increasing HDL levels. Gemfibrozil was shown in the primary prevention Helsinki Heart Study to decrease CAD events about 34% compared with placebo. Patients with elevated LDL and TG appeared to respond the best. The most common side effects of fibrates are upset stomach, nausea or vomiting. Abdominal pain is the second most common side effect. There is a slightly increased risk of gallstones. Gemfibrozil can exacerbate with effects of drugs that prevent blood clotting (anticoagulants), causing bleeding.

Omega-3 fatty acids inhibit the production of TG in liver by several postulated mechanisms, including interfering with the incorporation of FFA into TG (◘ Fig. 32.1). The omega-3 fatty acids are enriched in ecosapentanoic acid (EPA) and docosahexaenoic acid (DHA), which are concentrated from fish oils to a 90% purified form that is available in a prescription formulation, Lovaza. TG can be lowered by up to 50% at a dose of two, 1-g capsules taken twice daily.

32.7.6 Combination Pharmacotherapy

Statin therapy is most often started initially in those with CAD or CAD risk equivalence. Depending on the LDL-C response, it may be necessary to add a second drug to achieve the LDL-C goal, particularly the optional goal of 70 mg/dl (◘ Table 32.8). A second drug may also be necessary because of a low HDL-C, a high TG, or both. Statins have been used in combination with BAS, fibrates, niacin or a CAI. Sequestrants have been paired with fibrates, niacin and ezetimibe. Niacin and fibrates have also been used together. There are ongoing studies of ezetimibe combined with either niacin or fibrates. Different combination therapies may be required either because a patient is unable to tolerate the side effects of a particular class of drug or because a certain combination has not achieved optimal control of LDL-C, HDL-C, non-HDL-C or TG. Combination therapy has significant theoretical potential to reduce CAD further. The final answer to this hypothesis awaits the results of several large clinical trials.

32.8 Abbreviations

ABC	ATP-binding cassette
ACAT	acyl coenzyme A: cholesterol acyltransferase
Apo	apolipoprotein
ARH	autosomal recessive hypercholesterolaemia
ASP	acylation-stimulatory protein
BAS	bile acid sequestrant
BP	basic proteins
CAD	coronary artery disease
CAI	cholesterol absorption inhibitor
CESD	cholesteryl ester storage disease
CETP	cholesteryl ester transfer protein
FC	free cholesterol
FCHL	familial combined hyperlipidaemia
FDB	familial defective apoB-100
FFA	free fatty acids
FH	familial hypercholesterolaemia
FH3	heterozygous FH3
FHT	familial hypertriglyceridaemia
HDL	high-density lipoproteins
HDL-C	high-density lipoprotein cholesterol
HL	hepatic lipase
HMG-CoA	hydroxymethylglutaryl coenzyme A
HSCRP	highly sensitive C-reactive protein
IDL	intermediate-density lipoproteins
LAL	lysosomal acid lipase
LCAT	lecithin: cholesteryl acyltransferase
LDL	low-density lipoproteins
LDL-C	low-density lipoprotein cholesterol
LPL	lipoprotein lipase
LRP	LDL receptor-related protein
MCT	medium-chain TG
MTP	microsomal triglyceride transfer protein
PHLA	post-heparin lipolytic activity
SREBP	sterol-regulating element-binding protein
TC	total cholesterol
TG	triglycerides
VLDL	very low-density lipoproteins
VLDL-C	very low-density lipoprotein cholesterol

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Disorders of Cholesterol Synthesis

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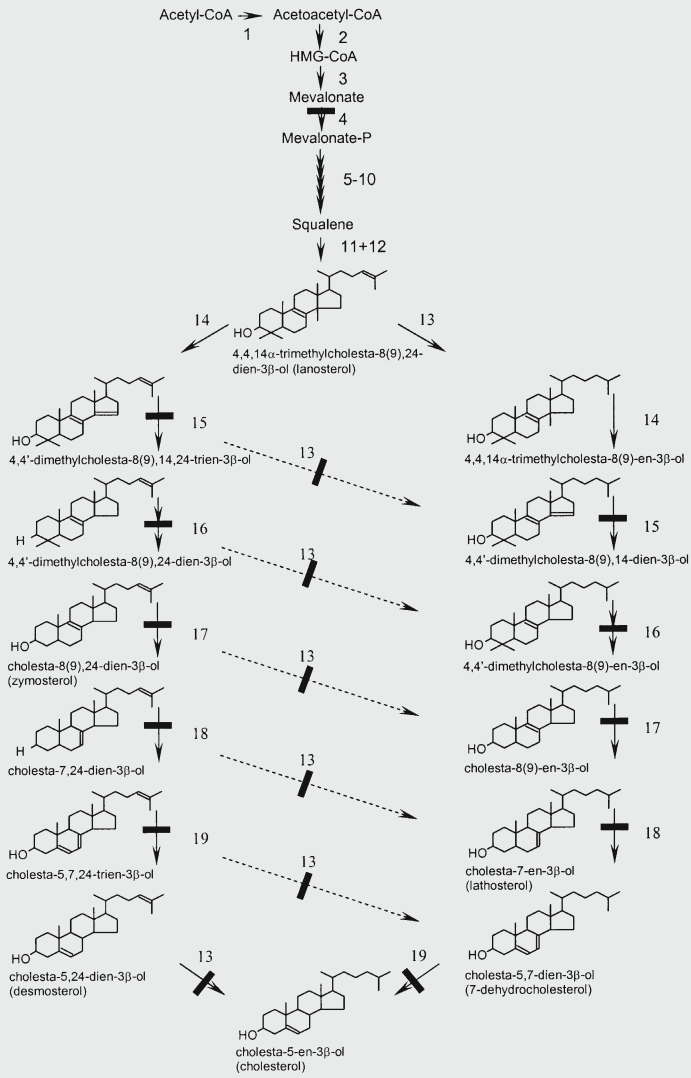
Cholesterol Synthesis

Cholesterol is synthesised by the isoprenoid biosynthetic pathway, which produces numerous biomolecules, i.e. 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 all subsequent isoprenoids (◘ Fig. 33.1). The first committed intermediate in the production of sterols is squalene, which after cyclisation is converted into lanosterol. Subsequent 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.



◘ **Fig. 33.1.** Pathway of isoprenoid and cholesterol synthesis. *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 Δ^{24}-reductase; **14**, sterol C-14 demethylase; **15**, sterol Δ^{14}-reductase; **16**, sterol C-4 demethylase complex; **17**, sterol Δ^{8}-Δ^{7} isomerase; **18**, sterol Δ^{5}-desaturase; **19**, sterol Δ^{7}-reductase. Enzyme deficiencies are indicated by *solid bars* across the *arrows*

Based on the finding of abnormally increased levels of intermediate sterol precursors in tissues and/or body fluids of patients followed by the demonstration of pathogenic mutations in genes encoding the implicated enzymes, seven distinct inherited disorders have been linked to specific enzyme defects in the isoprenoid/cholesterol biosynthetic pathway [2]. Only one of these disorders, i.e. mevalonate kinase deficiency, affects the synthesis of all isoprenoids. Patients with this disorder characteristically present with recurrent episodes of high fever and inflammation associated with abdominal pain, vomiting and diarrhoea, (cervical) lymphadenopathy, hepatosplenomegaly, arthralgia and skin rash, but may also have additional congenital anomalies.

The remaining six enzyme defects affect the synthesis of cholesterol and involve four autosomal recessive and two X-linked dominant inherited syndromes. Patients afflicted with one of these defects present with multiple congenital and morphogenic anomalies, including internal organ, skeletal and/or skin abnormalities, and/or a marked delay in psychomotor development reflecting cholesterol's pivotal role in human embryogenesis and development.

33.1 Mevalonate Kinase Deficiency

33.1.1 Clinical Presentation

Mevalonate kinase deficiency (MKD) includes the two previously defined clinical entities classic mevalonic aciduria (MA) and the more benign hyper-IgD and periodic fever syndrome (HIDS), which were both shown to be caused by markedly decreased activities of the enzyme mevalonate kinase (MK; enzyme 4 in ◘ Fig. 33.1). Patients with MKD suffer from characteristic episodes of high fever and inflammation that last 3-5 days, recur on average every 4-6 weeks and are associated with abdominal pain, vomiting and diarrhoea, (cervical) lymphadenopathy, hepatosplenomegaly, arthralgia and skin rash [3-5]. Peritoneal inflammatory adhesions leading to vomiting of bilious material, absence of bowel movements, and jejuno-ileal obstruction have also been described [6]. The onset of disease occurs mostly in the 1st year of life, often triggered by childhood vaccinations. The inflammatory episodes may be provoked by vaccinations, physical and emotional stress and minor trauma, but often occur without obvious reason. In addition to the inflammatory episodes, patients with the more severe MA phenotype can present with 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. The MA and HIDS phenotypes represent the severe and mild ends, respectively, of a clinical and biochemical continuum [7, 8].

33.1.2 Metabolic Derangement

The clinical presentation of patients with MK deficiency correlates well with the residual MK enzyme activity. In white blood cells or cultured primary skin fibroblasts from patients with the MA phenotype, the activity of MK is often below detection limits, while in cells from patients with the HIDS phenotype, a residual MK activity of 1-10% of the activities in cells from healthy controls is found [7-9].

MK catalyses the phosphorylation of mevalonate to produce 5-phosphomevalonate and is the next enzyme in the isoprenoid synthesis pathway after HMG-CoA reductase, the highly-regulated and major rate-limiting enzyme of the pathway [1]. As a consequence of the MK deficiency, high and moderately elevated levels of mevalonic acid can be detected in plasma and urine of patients with the MA and HIDS phenotype, respectively. Since MK functions relatively early in the biosynthetic pathway, the synthesis of all isoprenoids will be affected to a certain extent. Yet, most of the characteristic clinical manifestations are thought to be due to a (temporary) shortage of nonsterol isoprenoid end products [7]. It may well be possible, however, that in severe MA cases a relative shortage of sterol isoprenoids during embryonic development contributes to the congenital anomalies.

33.1.3 Genetics

MKD is autosomal recessively inherited and is due to mutations in the MK-encoding *MVK* gene located on chromosome 12q24 [8-10]. Nearly all patients with the HIDS phenotype are compound heterozygotes for the V377I *MVK* allele, which is found exclusively in patients with the HIDS phenotype, and a second allele, which is found also in patients with the MA phenotype [10]. Although the *V377I MVK* allele codes for an active MK enzyme, the correct assembly/maturation of the protein appears to be affected in a temperature-dependent manner, which explains the observed residual MK enzyme activity associated with the HIDS phenotype [10]. Other relatively common disease-causing mutations in the *MVK* gene are H20P, I268T and A334T. In total, more than 75 different pathogenic mutations have been identified that are widely distributed over the *MVK* gene [10] (unpublished data), most of which are listed in the infevers database at http://fmf.igh.cnrs.fr/infevers. These include primarily missense and nonsense mutations, while only a few insertions, deletions and splice site mutations have been identified.

33.1.4 Diagnostic Tests

Several diagnostic tools for laboratory analysis of MKD are available. 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) [11]. Owing to the variable degrees of MK deficiency, this test works best for patients with the MA phenotype, who have high levels of mevalonic acid (1-56 mol/mol creatinine in urine); it may not always be diagnostic for patients with the HIDS phenotype owing to their rather low levels even during fever (urinary concentration 0.005-0.040 mol/mol creatinine while normally not detectable). The best diagnostic tests remain the direct measurement of MK activities in white blood cells or primary skin fibroblasts from patients [12] and molecular analysis of the *MVK* gene through sequence analysis of the coding exons plus flanking intronic sequences [10]. The latter two tests are also used for prenatal diagnosis, which can be performed in chorionic villi, chorionic villous cells and amniotic fluid cells. Carrier detection is best performed by molecular testing.

33.1.5 Treatment and Prognosis

There is currently no efficacious treatment for MKD available. In some patients with the HIDS phenotype, clinical improvement as a result of treatment with corticosteroid, colchicine, or cyclosporin has been reported, but in the majority of patients these treatments do not have beneficial effects [5, 13]. Many patients have died in infancy with respiratory failure. In a small group of patients with the HIDS phenotype, simvastatin treatment led to a small decrease in the number of days of illness [14], but treatment with similar statins in MA patients led to worsening of the clinical symptoms. Treatment with etanercept, a soluble p75 TNF alpha receptor-Fc fusion protein, have been reported to lead to a reduction of the frequency and severity of symptoms in some patients with the HIDS phenotype [5]. Most promising results have been obtained by treatment with interleukin-1 (IL-1) receptor antagonists such as Anakinra, which blocks the biological activity (including inflammation) of IL-1 beta, an early pyrogenic cytokine that becomes elevated in MKD patients [5]. In a boy whose condition had failed to improve with anti-inflammatory treatment, HLA-identical allogeneic bone marrow transplantation resulted in remission of the febrile attacks and inflammation during a 15-month follow-up period [15].

The long-term outcome for patients with the HIDS phenotype is relatively benign as the clinical symptoms tend to become less frequent and less severe with age [5].

33.2 Smith-Lemli-Opitz Syndrome (7-Dehydrocholesterol Reductase Deficiency)

33.2.1 Clinical Presentation

Patients with Smith-Lemli-Opitz Syndrome (SLOS) clinically 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) [16-19]. Affected patients typically 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), postaxial polydactyly and short proximally placed thumbs. 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. For more detailed reports on this topic the reader is referred to other reviews summarizsing and discussing clinical aspects of SLOS [18, 19].

33.2.2 Metabolic Derangement

SLOS is caused by a deficiency of the enzyme 7-dehydrocholesterol reductase (DHCR7; sterol Δ^7-reductase; enzyme 19 in ◘ Fig. 33.1), which catalyses the reduction of the C7-C8 double bond of 7-dehydrocholesterol (cholesta-5,7-dien-3β-ol) to produce cholesterol (cholest-5-en-3β-ol), which is generally regarded as the predominant final step in cholesterol biosynthesis. As a consequence of the DHCR7 deficiency, low cholesterol and elevated levels of 7-dehydrocholesterol can be detected in plasma, cells and tissues of the vast majority of SLOS patients [20, 21]. In addition, elevated 8-dehydrocholesterol (cholesta-5,8(9)-dien-3β-ol) levels can be detected in plasma, probably synthesised 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. [22]). There is also evidence that the efficiency of transfer of cholesterol from mother to fetus may play a role in determining severity, as inferred from the significant correlation between patients' clinical severity scores and their mothers' apo E genotypes [23].

33.2.3 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 caucasians [19]. The higher incidences observed in particular in some East-European countries reflect founder effects.

The *DHCR7* gene encoding 3β-hydroxysterol Δ^7-reductase is located on chromosome 11q13 [24-26]. Currently, over 130 different pathogenic mutations have been reported in the *DHCR7* gene (for current listing see http://www.hgmd.cf.ac.uk/). Although mutations are distributed widely all over the gene, a few common mutations have been recognised, including T93M, R404C, W151X, V326I and IVS8-1G>C. By far the most prevalent in caucasians is the severe IVS8-1G>C splice site mutation (allele frequency of ~30%), which leads to aberrant splicing of the *DHCR7* mRNA, as a consequence of which no functional protein is produced.

33.2.4 Diagnostic Tests

Laboratory diagnosis of SLOS [21] 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. 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 through sequence analysis of the coding exons and flanking intronic sequences of the *DHCR7* gene is performed. A molecular test is the first choice for prenatal diagnosis, to be performed in chorionic villi, chorionic villous cells and amniotic fluid cells. Carrier detection is most reliably performed by molecular testing.

33.2.5 Treatment and Prognosis

It is generally considered that the availability of cholesterol during development of the fetus is one of the major determinants of the phenotypic expression in SLOS [19, 23]. Since most anomalies occurring in SLOS are of early-embryonic origin, it will 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 with or without bile acids (e.g. [27]). While this treatment leads to a substantial elevation of plasma cholesterol concentrations in patients, the plasma concentrations of 7-dehydrocholesterol and 8-dehydrocholesterol are often only marginally reduced. In general, the clinical effects of this treatment have been rather disappointing. Several early reports indicated that dietary cholesterol supplementation might improve behaviour, growth and general well-being in children with SLOS. However, a standardised study with 14 SLOS patients indicated that cholesterol supplementation had hardly any effect on developmental progress [28], and another showed there were no short-term improvements in behaviour [29]. Moreover, this treatment probably does not significantly change the sterol levels in brain, which are dependent on de novo cholesterol synthesis due to the limited ability of cholesterol to cross the blood-brain barrier. An alternative strategy, aimed primarily at lowering of the elevated 7-dehydrocholesterol and 8-dehydrocholesterol levels, has been the use of simvastatin, an oral HMG-CoA reductase inhibitor. Promising preliminary results were reported for mildly affected SLOS patients [30]. However, in studies conducted with larger numbers of patients no beneficial effects on either anthropometric measures or behaviour were seen [31].

33.3 X-Linked Dominant Chondrodysplasia Punctata 2 or Conradi-Hünermann Syndrome (Sterol Δ8-Δ7 Isomerase Deficiency)

33.3.1 Clinical Presentation

Patients with X-linked dominant chondrodysplasia punctata 2 (CDPX2), also known as Conradi-Hünermann or Happle syndrome, 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. These skin lesions are associated with cataracts and with skeletal abnormalities including short

stature, asymmetric rhizomelic shortening of the limbs, calcific stippling of the epiphyseal regions and craniofacial defects [32-34]. The pattern of the skin defects and probably also the variability in severity and asymmetry of the bone and eye abnormalities observed in CDPX2 patients are consistent with functional X-chromosomal mosaicism. The expression of these skin and skeletal abnormalities can be bilateral and is often asymmetric. As the defect is predominantly observed in females, CDPX2 is considered lethal in hemizygous males. However, several affected males with aberrant karyotypes, somatic mosaicism and even true hemizygous mutations have been identified.

33.3.2 Metabolic Derangement

CDPX2 is caused by a deficiency of the enzyme sterol Δ^8-Δ^7 isomerase (enzyme 17 in ◘ Fig. 33.1), which catalyses the conversion of cholesta-8(9)-en-3β-ol to lathosterol by shifting the double bond from the C8-C9 to the C7-C8 position [35-37]. 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, although the plasma cholesterol levels are often normal or low normal.

33.3.3 Genetics

CDPX2 is inherited as an X-linked dominant trait and is due to mutations in the *EBP* gene encoding the enzyme sterol Δ^8-Δ^7 isomerase located on chromosome Xp11.22-23 [35, 36]. The product of the *EBP* gene, i.e. emopamil binding protein, was initially identified as a binding protein for the Ca^{2+} antagonist emopamil and high-affinity acceptor for several other anti-ischaemic drugs, but later shown to encode for sterol Δ^8-Δ^7 isomerase. Currently, over 70 different disease-causing mutations have been identified in the *EBP* gene of primarily female patients with CDPX2 (for current listing see http://www.hgmd.cf.ac.uk/). Most analysed patients are heterozygous for a mutation that has arisen de novo (somatic mutations) in line with the sporadic nature of the disorder, but in a few cases indications for gonadal mosaicism have been obtained. Inheritance of a mutation from an affected mother usually results in a more severe expression of the disease in offspring.

33.3.4 Diagnostic Tests

Laboratory diagnosis of CDPX2 can be achieved by GC-MS analysis of plasma sterols of patients to detect cholesta-8(9)-en-3β-ol [37]. 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 by sequence analysis of the coding exons and flanking intronic sequences of the *EBP* gene. Prenatal diagnosis by molecular analysis is possible [38].

33.3.5 Treatment and Prognosis

Long-term outcome of patients with CDPX2 depends on the severity of clinical symptoms. Surviving male patients usually show severe developmental delay. In contrast, 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 [39].

33.4 CHILD Syndrome (3β-Hydroxysteroid C-4 Dehydrogenase Deficiency)

33.4.1 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 [34, 40]. 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. Unlike those with 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 hemizygous males, as so far hardly any males with the defect have been diagnosed.

33.4.2 Metabolic Derangement

CHILD syndrome is caused by a deficient activity of a 3β-hydroxysteroid dehydrogenase [41]; it has been suggested that this is part of a sterol C-4 demethylase complex

(composed of a C-4 methyl oxidase, a 4α-carboxysterol-C-4 dehydrogenase [i.e. 3β-hydroxysteroid dehydrogenase] and a C-4 ketoreductase [enzyme complex 16 in ◘ Fig. 33.1]) which catalyses the sequential removal of the two methyl groups at the C4 position of early sterol precursors (e.g. lanosterol). 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.

33.4.3 Genetics

CHILD syndrome is inherited as an X-linked dominant trait due to heterozygous mutations in the *NSDHL* gene encoding 3β-hydroxysteroid dehydrogenase and located on chromosome Xq28 [41, 42]. In one patient clinically diagnosed with CHILD syndrome, a heterozygous mutation was identified in the *EBP* gene [43]. Currently, over 20 different pathogenic mutations have been identified in the *NSDHL* gene (for current listing see http://www.hgmd.cf.ac.uk/).

33.4.4 Diagnostic Tests

The only diagnostic test for CHILD syndrome is mutation analysis by sequencing the coding exons and flanking intronic sequences of the *NSDHL* gene. If no mutation is found in the *NSDHL* gene, one should consider also sequencing the *EBP* gene, as mutations in this gene also have been linked to CHILD syndrome [43].

33.4.5 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.

33.5 Desmosterolosis (Desmosterol Reductase Deficiency)

33.5.1 Clinical Presentation

Currently five patients with desmosterolosis have been identified, only two of whom have been reported. 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 and generalised osteosclerosis [44]. The second reported infant is 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 [45]. Comparison with the clinical manifestations of the other three unpublished but deceased patients indicates that dysmorphic facial features, contractures and brain malformations, including agenesis of the corpus callosum, are prominent features in desmosterolosis.

33.5.2 Metabolic Derangement

Desmosterolosis is due to a deficiency of the enzyme sterol Δ^{24}-reductase (desmosterol reductase; enzyme 13 in ◘ Fig. 33.1), which catalyses the reduction of the Δ^{24} double bond of sterol intermediates (including desmosterol) in cholesterol biosynthesis [46]. As a consequence, elevated levels of the cholesterol precursor desmosterol can be detected in plasma, tissue and cultured cells of patients with desmosterolosis [44-46].

33.5.3 Genetics

Desmosterolosis is an autosomal recessive disorder due to mutations in the *DHCR24* gene encoding 3β-hydroxysterol Δ^{24}-reductase and located on chromosome 1p31.1-p33. Sequence analysis of the *DHCR24* gene of the five patients revealed seven different pathogenic mutations [46] (unpublished data).

33.5.4 Diagnostic Tests

Laboratory diagnosis of desmosterolosis includes sterol analysis of plasma, tissues or cultured cells by GC-MS (detection of desmosterol) and mutation analysis by sequencing the coding exons and flanking intronic sequences of the *DHCR24* gene [46].

33.5.5 Treatment and Prognosis

Of the five identified patients, four patients died in early infancy. Only one patient survived, but showed profound developmental delay.

33.6 Lathosterolosis (Sterol Δ5-Desaturase Deficiency)

33.6.1 Clinical Presentation

Only two patients with lathosterolosis have been reported. 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 [47]. The second patient was a boy who presented at birth 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 [48].

33.6.2 Metabolic Derangement

Lathosterolosis is due to a deficiency of the enzyme sterol Δ^5-desaturase (enzyme 18 in ◘ Fig. 33.1), which introduces the C5-C6 double bond in lathosterol to produce 7-dehydrocholesterol, the ultimate precursor of cholesterol [47, 48]. As a consequence, elevated levels of lathosterol (and lowered cholesterol) can be detected in plasma, (tissue) and cultured cells of patients with lathosterolosis.

33.6.3 Genetics

Lathosterolosis is an autosomal recessive disorder due to mutations in the *SC5D* gene encoding 3β-hydroxysterol Δ^5-desaturase and located on chromosome 11q23.3. Sequence analysis of the *SC5D* gene of the two patients revealed three different pathogenic mutations [47, 48].

33.6.4 Diagnostic Tests

Laboratory diagnosis of lathosterolosis includes sterol analysis of plasma, tissues or cultured cells by GC-MS (detection of lathosterol) and mutation analysis by sequencing the coding exons and flanking intronic sequences of the *SC5D* gene [47, 48].

33.6.5 Treatment and Prognosis

No information on treatment and long-term outcome is available, but it is possible that in some cases treatment for chronic cholestatic liver disease (e.g. fat-soluble vitamin supplementation) will be required.

33.7 Hydrops – Ectopic Calcification – Moth-eaten (HEM) Skeletal Dysplasia or Greenberg Skeletal Dysplasia (Sterol Δ14-Reductase Deficiency)

33.7.1 Clinical Presentation

HEM skeletal dysplasia, also known as Greenberg skeletal dysplasia, is a rare syndrome 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 nonskeletal malformations [49-51].

HEM skeletal dysplasia is allelic to Pelger-Huet anomaly [51-54], 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 Pelger-Huet anomaly do not show any evident clinical symptoms, but few (presumed) homozygotes for this defect with variable minor skeletal abnormalities and developmental delay have been reported [52].

33.7.2 Metabolic Derangement

HEM skeletal dysplasia is due to a deficiency of the enzyme sterol Δ^{14}-reductase (enzyme 15 in ◘ Fig. 33.1), which catalyses the reduction of the Δ^{14} double bond in early sterol intermediates [52-54]. As a consequence, elevated levels of cholesta-8,14-dien-3β-ol (and minor levels of cholesta-8,14,24-trien-3β-ol) can be detected in

tissues and cells of fetuses with HEM skeletal dysplasia. Heterozygous individuals with Pelger-Huet anomaly do not show aberrant sterol precursors.

33.7.3 Genetics

HEM skeletal dysplasia is an autosomal recessive disorder due to mutations in the *LBR* gene encoding lamin B receptor and located on chromosome 1q42 [52]. Lamin B receptor consists of an N-terminal lamin B/DNA-binding domain of ~200 amino acids followed by a C-terminal sterol reductase-like domain of ~450 amino acids, which exhibits the sterol Δ^{14}-reductase activity.

More than six pathogenic mutations have been detected in the *LBR* gene of fetuses affected with HEM dysplasia (unpublished data, but for current listing see also http://www.hgmd.cf.ac.uk/).

In addition, several heterozygous splice-site, frameshift and nonsense mutations have been detected in the *LBR* gene of individuals with Pelger-Huet anomaly [51]. The demonstration of Pelger-Huet anomaly in one of the parents of a fetus affected with HEM skeletal dysplasia confirms that Pelger-Huet anomaly represents the heterozygous state of 3β-hydroxysterol Δ^{14}-reductase deficiency [52-54]. However, Pelger-Huet anomaly is not seen in a parent carrying a missense mutation affecting only the Δ^{14}-reductase region of the *LBR* gene [54].

33.7.4 Diagnostic Tests

Fetuses affected with HEM skeletal dysplasia are often detected by fetal ultrasound examination. Pelger-Huet anomaly can be diagnosed by microscopy of peripheral blood smears. Laboratory diagnosis of HEM skeletal dysplasia includes sterol analysis of tissues or cells by GC-MS (detection of cholesta-8,14-dien-3β-ol). Molecular analysis includes sequencing of the coding exons and flanking intronic sequences of the *LBR* gene [51, 52]. Prenatal testing has been performed by molecular testing [50].

33.7.5 Treatment and Prognosis

Most cases of Greenberg skeletal dysplasia terminate in early embryonic stages (10-20 weeks of gestation). One adult individual diagnosed with Pelger-Huet anomaly and homozygous for a splice-site mutation in the *LBR* gene has been described with developmental delay, macrocephaly and a ventricular septal defect. No information is available, however, on the effect of the mutation on cholesterol biosynthesis in this individual, if any.

33.8 Other Disorders

Accumulation of lanosterol has been described in some patients diagnosed with Antley-Bixler syndrome, suggesting a defect of lanosterol C14-demethylase. However, no mutations in *CYP51,* the gene encoding lanosterol C14-demethylase, have yet been described. Instead, it appears that a reduced activity of this enzyme (as well as enzymes of steroidogenesis) may occur as a result of mutations in the *POR* gene encoding cytochrome P450 oxidoreductase [55].

CK syndrome (CKS) is an X-linked recessive intellectual disability syndrome characterised by dysmorphism, cortical brain malformations and an asthenic build. In the first reported family, a 3-bp deletion in exon 7 of NAD(P)-dependent steroid dehydrogenase-like (NSDHL) has recently been identified, and mutations in this gene have been also found in males of another reported family with a CKS. As described for the allelic disorder CHILD syndrome, cells and cerebrospinal fluid from CKS patients have increased methyl sterol levels [56].

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Disorders of Bile Acid Synthesis

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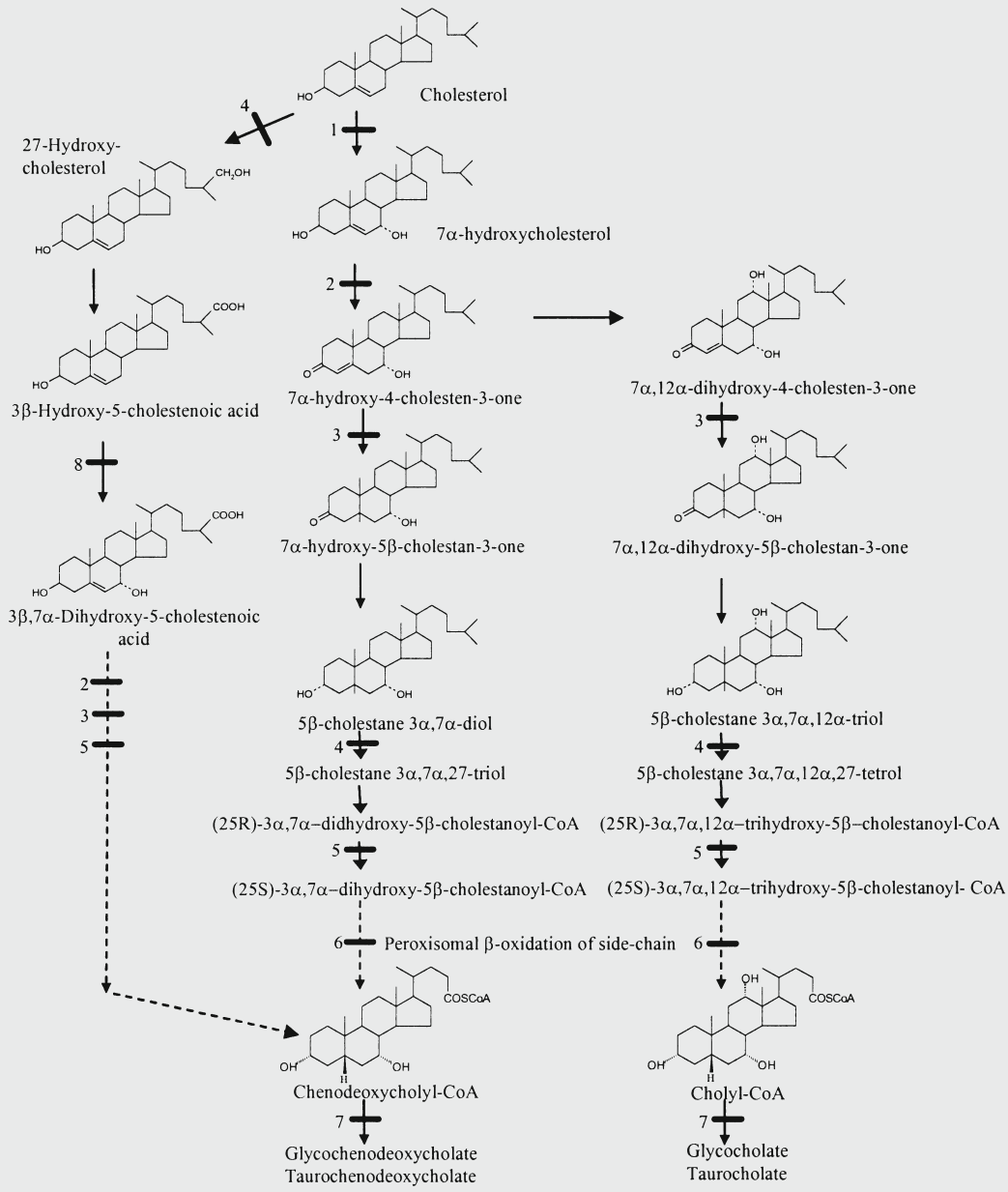
Bile Acid Synthesis

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. 34.1); 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.



◘ **Fig. 34.1.** Major reactions involved in the synthesis of bile acids from cholesterol. **1**, Cholesterol 7α-hydroxylase; **2**, 3β-hydroxy-Δ^5-C_{27}-steroid dehydrogenase/isomerase; **3**, Δ^4-3-oxosteroid-5β-reductase; **4**, sterol 27-hydroxylase; **5**, α-methylacyl-CoA racemase; **6**, enzymes ofperoxisomal biogenesis and β-oxidation; **7**, bile acid-CoA: amino acid *N*-acyl transferase; **8**, oxysterol 7α-hydroxylase. Enzyme defects are depicted by *solid bars* across the *arrows*

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. CTX may present with cataracts and mental retardation in childhood, followed by motor dysfunction and tendon xanthomata in the second or third decade. Death may be caused by progressive motor dysfunction and dementia or by premature atherosclerosis. Chenodeoxycholic acid has been shown to halt or even reverse neurological dysfunction. α-Methyl-acyl-CoA racemase deficiency can produce developmental delay, epilepsy, acute encephalopathy, tremor, pigmentary retinopathy, hemiparesis, spastic paraparesis and peripheral neuropathy. Other inborn errors of bile acid synthesis include *oxysterol 7α-hydroxylase deficiency* (rapidly progressive liver disease in infancy or later onset hereditary spastic paraparesis with diminished vibration sensation and proprioception), 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 41.

34.1 Introduction

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. Because of the broad specificity of many of the enzymes, the major metabolites are not those immediately proximal to the block. For instance, in 3β-hydroxy-Δ^5-C_{27}-steroid dehydrogenase deficiency (enzyme 2 in ◘ Fig. 34.1) 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.

34.2 3β-Hydroxy-Δ5-C27-Steroid Dehydrogenase Deficiency

34.2.1 Clinical Presentation

3β-Dehydrogenase deficiency was first described in 1987 [1]. Reviews of 38 children diagnosed in Cincinnati and 18 cases diagnosed in London were published in 2007 and 2010, respectively [2,3]. 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). 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 [4]. 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 in the second decade of life has also been described [2].

34.2.2 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. 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 [5]. 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. 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.
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.

34.2.3 Genetics

3β-Dehydrogenase deficiency is an autosomal-recessive trait caused by mutations in the *HSD3B7* gene 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 of the gene (Δ1057–1058) [6]. In 2003, Cheng et al. reported mutations in 15 additional patients from 13 kindreds with 3β-dehydrogenase deficiency [7]. In patients with neonatal cholestasis, they identified deletions (310delC, 63delAG), a splice site mutation (340+1 G>T) and a missense mutation (E147K).

34.2.4 Diagnostic Tests

The diagnosis is established by demonstrating the presence of the characteristic Δ^5 bile acids 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. They may be destroyed by some of the methods that are used for solvolysis of sulphated bile acids prior to chromatographic analysis. Solvolysis is best performed using tetrahydrofuran/methanol/trifluoroacetic acid (900:100:1 volume ratio) [3, 5]. Analysis by fast-atom-bombardment mass spectrometry (FAB-MS) overcomes the problem of lability [1, 8]. More recently, because electrospray ionisation tandem mass spectrometry (ESI-MS/MS) is in use in many laboratories, diagnostic bile acids have been detected using this methodology [9].

- **Plasma**

If plasma bile acids are analysed using a gas chromatography (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/MS [9].

- **Urine**

Urine analysed by negative ion FAB-MS shows the characteristic ions of the diagnostic unsaturated bile acids: mass/charge ratios (m/z) = 469, 485, 526 and 542. Using electrospray ionisation tandem mass spectrometry, 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.

- **Fibroblasts**

3β-Dehydrogenase can be assayed in cultured skin fibroblasts using tritiated 7α-hydroxycholesterol [10]. Patients show very low activity.

34.2.5 Treatment and Prognosis

Emergency treatment of coagulopathy with parenteral vitamin K may be required [3]. 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, 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 that 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 [1]. 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 [3]. Gonzalez et al. have reported treatment with cholic

acid alone in 15 patients with 3β-HSDH deficiency. They described normalisation of physical examination findings, laboratory test results and liver ultrasound, and improvement in liver biopsy appearances [11]. Two women had normal pregnancies during treatment.

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).

34.3 Δ4-3-Oxosteroid 5β-Reductase Deficiency

34.3.1 Clinical Presentation

Patients who excrete 3-oxo-Δ^4 bile acids as the major urinary bile acids can be divided into three groups – those who have proven mutations in *SRD5B1* (*AKR1D1*, the gene encoding the 5β-reductase enzyme) [12-14]; those in whom this has been excluded [15]; and those in whom the results of gene analysis have not been published [16, 17]. In the last two 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 [18, 19], the description in this chapter will focus on the five patients with proven 5β-reductase mutations.

In two of the four families described, the parents were consanguineous [12-14]. All five patients presented in the neonatal period with cholestatic jaundice with raised transaminases but normal γ-GT, low vitamin E and prolonged clotting times, which improved with parenteral vitamin K. Liver biopsies showed giant cell transformation, canalicular and hepatocellular cholestasis, portal inflammation, septal fibrosis, occasional necrotic foci and, in some cases, increased extramedullary haemopoiesis. Without treatment, cholestasis persisted in all cases.

34.3.2 Metabolic Derangement

Mutations in *SRD5B1* lead to reduced activity of the hepatic enzyme 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 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 [20].

34.3.3 Genetics

Primary 5β-reductase deficiency is an autosomal recessive disorder caused by mutations in *SRD5B1* (*AKR1D1*, the gene encoding the 5β-reductase enzyme) [13, 14]. The mutations that have been described are 385 C>T (missense), 467 C>G (missense), 511delT (frameshift, premature stop codon), 662C>T (missense) and 850 C>T (missense).

34.3.4 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 [12].

- **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.

34.3.5 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 (for at least 8 years) good health. Successful regimens include chenodeoxycholic acid plus cholic acid (8 mg/kg/day of each) [12, 13] and cholic acid alone (initially 13 mg/kg/day, subsequently 6 mg/kg/day) [14]. Thus, of the patients with proven 5β-reductase mutations, one infant progressed to liver failure, failed to respond to treatment with ursodeoxycholic acid or chenodeoxycholic acid and had a liver transplantation at 19 weeks. One child failed to respond to ursodeoxycholic acid treatment but responded extremely well to treatment with chenodeoxycholic acid and cholic acid (started at 8 months) and was asymptomatic at the age of 10 years. One patient showed an initial response to chenodeoxycholic acid plus cholic acid but then deteriorated (possibly due to cytomegalovirus infection) and required transplantation. Twin patients responded to treatment with cholic acid started at 8 months, and they were well at 5 years.

34.4 Cerebrotendinous Xanthomatosis (Sterol 27-Hydroxylase Deficiency)

34.4.1 Clinical Presentation

Cholestatic jaundice in infancy may be the first manifestation of cerebrotendinous xanthomatosis (CTX) [21, 22]. However, it usually improves spontaneously. 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. [23] 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 [24] (▶ 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 [25]. 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 [26]. Patients with untreated CTX usually die from progressive neurological dysfunction or myocardial infarction between the ages of 30 years and 60 years.

34.4.2 Metabolic Derangement

CTX is caused by a defect in the gene for sterol 27-hydroxylase, 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 [27]. 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 major symptoms of CTX are produced by accumulation of cholestanol (and cholesterol) in almost every tissue of the body, particularly in the nervous system, atherosclerotic plaques and tendon xanthomata.

Sterol 27-hydoxylase 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 [28]. Disruption of this pathway in CTX provides a further explanation for the accumulation of cholesterol in the tissues.

34.4.3 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 [27]. CTX can be caused by point mutations that lead to production of an inactive enzyme (R362C and R446C) [29]. 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 [30]. Many other mutations have now been described [31].

34.4.4 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. 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 [32]. 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) [33]. The urinary bile-alcohol excretion following cholestyramine administration has been used as a test for carriers of CTX [34].

- **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 [35].

- **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 (seeabove).

34.4.5 Treatment and Prognosis

The results of treatment with chenodeoxycholic acid were first reported in 1984 [36]. 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 [37]. 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) [38] and low-density lipoprotein apheresis [39]. There is insufficient information available to assess these forms of treatment at the present time. The osteoporosis seen in patients with CTX appears to be resistant to chenodeoxycholic acid therapy [40]. 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 [21, 22].

34.5 α-Methylacyl-CoA Racemase Deficiency

34.5.1 Clinical Presentation

α-Methylacyl-CoA racemase (AMACR) deficiency was first described in 2000 [41]. 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 and cognitive decline [41-43]. 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 [44, 45]. 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.

34.5.2 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. 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.

34.5.3 Genetics

AMACR deficiency is caused by mutations in the *AMACR* gene on chromosome 5p13.2–5q11.1. Pathogenic mutations in the adults with neurological disease included a common mutation, S52P, and L107P [41-43]. The S52P mutation was also found in the siblings who presented with neonatal coagulopathy [45].

34.5.4 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.

34.5.5 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. [44, 45]. 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.

34.6 Oxysterol 7α-Hydroxylase Deficiency

34.6.1 Clinical Presentation

Oxysterol 7α-hydroxylase was first described in a 10-week-old male infant with severe cholestasis, cirrhosis and liver synthetic failure [46]. A second patient was also jaundiced from early infancy and died of liver failure at 11 months [47]. We have diagnosed an infant who presented with liver failure and hypoglycaemia at 3 months but recovered completely with chenodeoxycholic acid treatment [48]. Oxysterol 7α-hydroxylase deficiency has also been identified as a cause of a recessive form of hereditary spastic paraplegia (HSP) [49, 50]. 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.

34.6.2 Metabolic Derangement

This recessive disorder is due to mutations in the gene encoding microsomal oxysterol 7-hydroxylase, 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. In addition the metabolism of neurosteroids is affected as the oxysterol 7-hydroxylase catalyses the conversion of dehydroepiandrosterone (DHEA) to 7-hydroxy-DHEA.

34.6.3 Genetics

The two reported children with liver disease were homozygous for nonsense mutations, R388X and R112X, respectively, in the *CYP7B1* gene on chromosome 8q21.3.

The patients with HSP were mostly homozygous for missense mutations in the gene: S363F, F216S, G57R and R417H. However, one individual with HSP had the R388X nonsense mutation.

34.6.4 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. 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 [46].

34.6.5 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 to treatment with chenodeoxycholic acid and is well at 3 years of age. Obviously, some patients must never develop significant liver disease and present later with HSP.

34.7 Bile Acid Amidation Defect 1: Bile Acid CoA: Amino Acid N-Acyl Transferase Deficiency

34.7.1 Clinical Presentation

Bile acid CoA: amino acid *N*-acyl transferase (BATT) 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 [51]. 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.

34.7.2 Metabolic Derangement

Without the enzyme bile acid coenzyme A: amino acid *N*-acyl transferase, encoded by the *BAAT* gene, 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.

34.7.3 Genetics

Defective amidation of bile acids in the Amish is caused by homozygosity for a missense mutation (226 A>G; M76V) in the *BAAT* gene. Our patient was homozygous for a nonsense mutation (R139X).

34.7.4 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).

34.7.5 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 but it is important to note that familial hypercholanaemia in the Amish can be caused by defects in a gene responsible for integrity of tight junctions (*TJP2*) as well as by mutations in the *BAAT* gene.

34.8 Bile Acid Amidation Defect 2: Bile Acid CoA Ligase Deficiency

34.8.1 Clinical Presentation

The Cincinatti group described a 14-year-old boy of Laotian descent with a defect in bile acid amidation presumed to involve the bile acid-CoA ligase, who presented in the first 3 months of life with unconjugated hyperbilirubinaemia, elevated serum transaminases with normal γ-glutamyltranspeptidase and fat and fat-soluble vitamin malabsorption (vitamin K deficiency and rickets) [52]. The same group has since identified further patients with neonatal cholestasis, growth failure or fat-soluble

vitamin malabsorption who they believe have the same enzyme defect [2]. These patients excrete large amounts of unconjugated cholic acid in the urine. We have identified a 3-year-old female infant who is the only child of parents of Pakistani origin. She was born at 27 weeks' gestation and required a prolonged period of parenteral nutrition. She developed conjugated hyperbilirubinaemia, which persisted until the age of 12 months. A liver biopsy showed portal to portal bridging fibrosis. Between 12 months and 18 months of age liver function tests returned to normal. This patient has been shown to have mutations in the gene encoding the bile acid-CoA ligase (*SLC27A5*) [53].

34.8.2 Metabolic Derangement

De novo synthesis of bile acids generates the CoA esters of chenodeoxycholic acid and cholic acid, so that the bile acid CoA ligase active on these bile acids is not thought to play a major role in de novo synthesis. However, 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. 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.

34.8.3 Genetics

In our patient, no mutations were found in the *BAAT* gene; however, sequence analysis of *SLC27A5* showed that she was homozygous for a mutation in this gene – His338Tyr; c.1012c>t, which is in a highly conserved area of the gene, and which is probably important for protein activity.

34.8.4 Diagnostic Tests

The urine bile acid profile is similar to that seen in BAAT deficiency, showing the following compounds: nonamidated cheodeoxycholic 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.

34.8.5 Treatment and Prognosis

Treatment with oral glycocholic acid is under investigation [54]. This will need careful evaluation in view of the possibility of spontaneous improvement in early infancy.

34.9 Cholesterol 7α-Hydroxylase Deficiency

34.9.1 Clinical Presentation

Homozygous cholesterol 7α-hydroxylase deficiency has been detected in three adults with hypercholesterolaemia, hypertriglyceridaemia and premature gallstone disease [55]. 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 codominant disorder.

34.9.2 Metabolic Derangement

Cholesterol 7α-hydroxylase 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.

34.9.3 Genetics

Cholesterol 7α-hydroxylase deficiency is caused by mutations in the *CYP7A1* gene. The only mutation described to date is a frameshift mutation (L413fsX414) that results in loss of the active site and enzyme function.

34.9.4 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.

34.9.5 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.

34.10 Disorders of Peroxisome Biogenesis and Peroxisomal β-Oxidation

These conditions are described in ►Chapter 41. Neurological disease usually dominates the clinical picture, but some children with Zellweger syndrome or infantile Refsum's disease have quite marked cholestatic liver disease.

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Disorders of Phospholipid and Glycosphingolipid Synthesis

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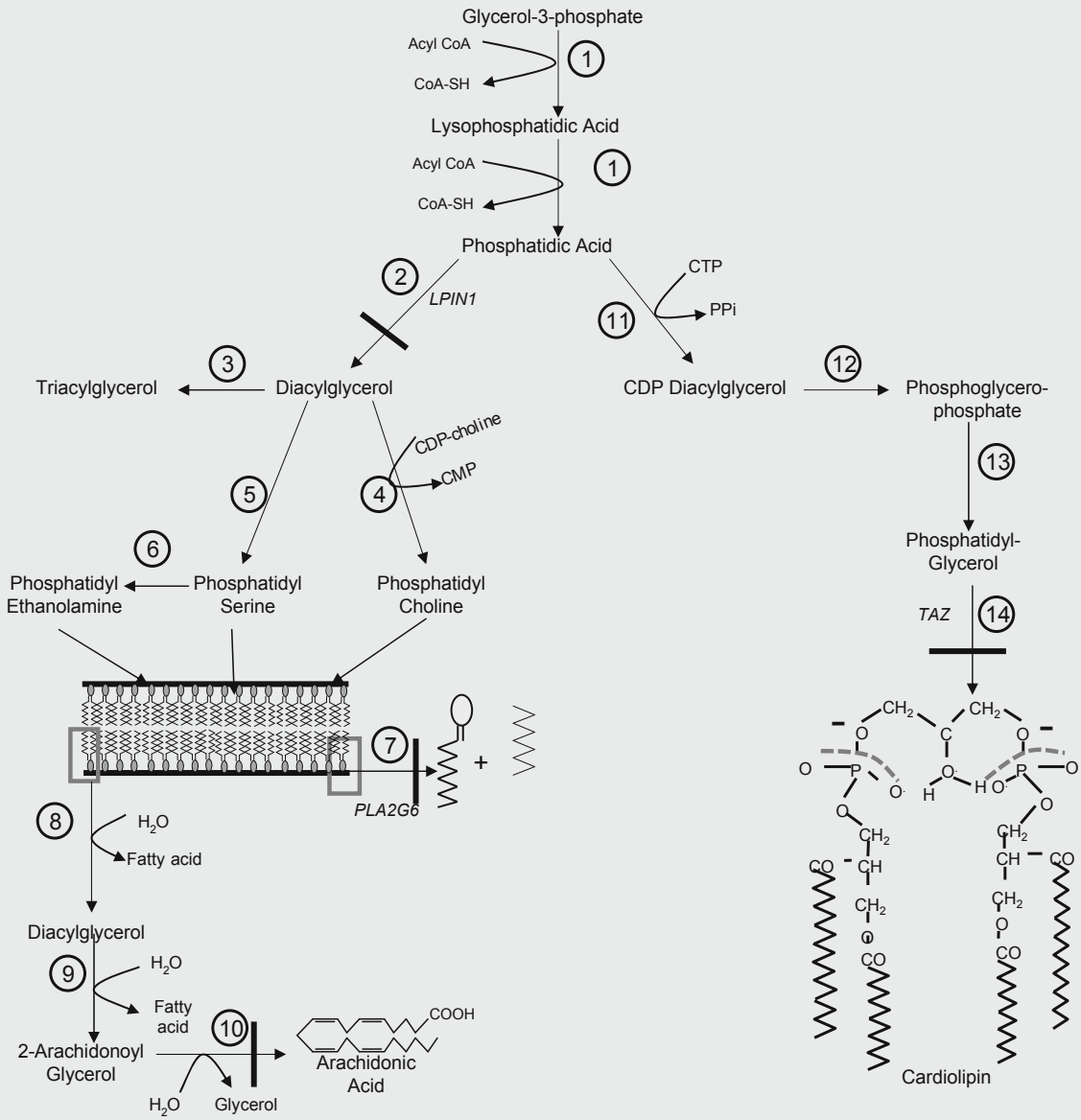
Phospholipid Biosynthesis

Phospholipids are components of cell membranes, composed of a phosphate group that constitutes an hydrophylic head, located on the surface of a bilayer, and a fatty acid chain that forms an hydrophobic tail, located inside (◘ Fig. 35.1). They are synthesised de novo from glycerol-3-phosphate formed from glycerol or from the glycolytic intermediate dihydroxyacetone phosphate. Glycerol-3-phosphate is first esterified twice with acyl CoA by acyl CoA transferase. This



◘ **Fig. 35.1.** Major reactions involved in phospholipid biosynthesis: *CoA*, coenzyme A; *CDP*, cytidyldiphosphate; *CMP*, cytidylmonophosphate; *CTP*, cytidyltriphosphate; *TAZ*, tafazzin. **1**, Acyl-CoA transferase; **2**, phosphatidic acid phosphatase; **3**, diacylglycerolacetyltransferase; **4**, phosphatidylcholine synthase; **5**, phosphatidylserine synthase; **6**, phosphatidylserine decraboxylase; **7**, phospholipase A2β; **8**, phospholipase Cγ; **9**, diacylglycerol lipase; **10**, α-/β-hydroxylase (ABDH12); **11**, phosphatidic acid cytidyltransferase; **12**, phosphatidic acid glycerolphosphate synthase; **13**, phosphatase; **14**, cardiolipin synthase. Enzyme deficiencies are indicated by *solid bars* across the *arrows*

results in the formation of lysophosphatidic acid, followed by that of phosphatidic acid. Phosphatidic acid can be converted either into diacylglycerol by phosphatidic acid phosphatase, or into CDP diacylglycerol by phosphatidic acid cytidyltransferase. Diacylgycerol is an essential intermediate for the synthesis of triglycerides and of various phosphoglycerides: phosphatidyl choline, phosphatidyl serine, phosphatidyl ethanolamine (◘ Fig. 35.1) and phosphatidyl inositol (not shown).

Diacylglycerol can also be formed from membrane phospholipids by phospholipase C and converted by diacylglycerol lipase into 2-arachidonoyl glycerol, which can be hydrolysed into arachidonic acid by alpha beta hydrolase 12. Phospholipids can also release free fatty acids in a reaction catalysed by phospholipase A2. CDP diacylglycerol is converted by the sequential action of phosphatidic acid glycerol phosphate synthase and phosphatase into phosphatidylglycerol. This compound is the precursor of cardiolipin, an important phospholipid of the mitochondrial membrane. Its formation as a mature, symmetrical form requires a recently identified remodelling enzyme, monolysocardiolipin acyl transferase.

Recent advances in instrumentation and technology have led to the identification of seven inherited metabolic diseases linked to a defect in phospholipid and glycosphingolipid biosynthesis. Analysis of patients' plasma lipidomes can provide a valuable approach for detecting and monitoring diseases and the efficacy of their treatment [1].

Phosphatidic acid phosphatase (lipin-1) deficiency should be regarded as a major cause of severe myoglobinuria in infancy. This enzyme catalyses the conversion of phosphatidic acid to diacylglycerol. Mutations of the *LIPN1* gene are transmitted as autosomal recessive traits.

Cardiolipin remodelling enzyme deficiency caused by *Tafazzin* gene mutations causes *Barth syndrome*, an X-linked recessive disease. Patients have variable clinical findings, often including cardiomyopathy, heart failure, myopathy, neutropenia and growth retardation.

Phospholipase A2 deficiency as a result of *PLA2G6* mutations is responsible for three different neurological phenotypes: (i) infantile neuroaxonal dystrophy (INAD); (ii) neurodegeneration with brain iron accumulation (NBIA), and (iii) dystonia-Parkinsonism in adults. *PLA2G6* mutations are transmitted as autosomal recessive traits.

The deficiency of α/β-hydrolase12 (ABHD12), which has recently been shown to hydrolyse 2-arachidonoyl glycerol, is responsible for the *PHARC syndrome*, a neurodegenerative disease marked by early-onset cataract, hearing loss, retinitis pigmentosa, demyelinating sensorimotor polyneuropathy and cerebellar ataxia.

35.1 Disorders of Phospholipid Synthesis

35.1.1 LIPN1 Deficiency (Phosphatidate Phosphatase 1 Deficiency)

- **Clinical Presentation**

All patients suffer from recurrent episodes of myoglobinuria precipitated by febrile illnesses or occasionally by anaesthesia or fasting and lasting 7–10 days [2]. In a recent study involving 29 patients from 23 families the number of acute episodes ranged from 1 to 10 per patient [3]. The first episode occurs between the 2nd birthday and the age of 7 years. The presenting symptoms are generalised weakness, inability to walk, myalgia and dark urine, and on examination the thigh and calf muscles are very tender. Lower limb muscle strength is markedly reduced, and the patellar and Achilles reflexes are absent; patients are unable to lift their legs against gravity but can withdraw them in response to painful stimuli. The strength, tone and reflexes of the upper limbs are usually preserved. The central nervous system is typically spared; however, when first admitted at age 18 months, one patient had generalised muscle hypotonia. Plasma creatine kinase (CK) levels are markedly elevated (peak levels 180,000-450,000 units, normal <150) with overt myoglobinuria. Lactate dehydrogenase and aspartate aminotransferase are also raised (peak levels 24,540 units/l and 12,620 units/l, respectively). Uric acid, blood gases, plasma lactate, total and free carnitine, blood acylcarnitine profile, plasma amino acids, beta oxidation of labelled palmitate, CPT2 activity in lymphocytes and urinary organic acids are normal. Normal results are also obtained by interictal electromyography, brain CT scan, abdominal ultrasound and echocardiogram. In a biopsy performed in one patient, 2 months after an episode of rhabdomyolysis the enzymatic activities

of the five mitochondrial respiratory-chain complexes and the pyruvate dehydrogenase complex were normal. The muscle contained no excess fat or glycogen, and the cytoarchitecture was preserved. Between episodes patients are healthy; their weight and height are in the normal range and plasma CK levels are normal to subnormal. Intellectual ability is reportedly average, but one patient suffered mild learning disability at 9 years of age [2].

▪ Metabolic Derangement

Lipin-1 exhibits a dual role: as a phosphatidate phosphatase 1 (PAP) for triacylglycerol and phospholipid biosynthesis (step 2 in ◘ Fig. 35.1) and as a transcriptional co-activator together with peroxisome proliferator-activated receptor-α (PPARα) and peroxisome proliferator-activated receptor coactivator-1α (PGC-1α) to regulate the expression of genes encoding fatty acid oxidation and respiratory chain enzymes [4, 5]. Lipin-1-related rhabdomyolysis could result from lysophosphatidic acid accumulation and the subsequent remodelling of membranes induced by phospholipid imbalances, as proposed in Barth syndrome [6, 7].

The clinical manifestations of *LPIN1* mutations in humans are confined to skeletal muscle. This restricted phenotype might be explained by the nearly ubiquitous expression of two other *LPIN* genes, *LPIN2* and *LPIN3* [4]. *LPIN1* expression is most prominent in skeletal muscle and adipose tissue. However, the human *LPIN2* is similarly expressed in adipose tissue, which probably accounts for the lack of lipodystrophy in LIPN1-deficient patients.

▪ Genetics

The *LPIN1* gene encodes lipin-1, a phosphatidic acid phosphatase that consists of 890 amino acids with two conserved domains. The mutations identified in the first three patients create a stop codon at residues 215, 388 and 800 or produce frameshifts as a result of exons being skipped, resulting in truncated proteins lacking catalytic activity [2]. In the subsequent series of 29 patients of various ethnic origins, 17 (59%) carried recessive nonsense or frameshift mutations, or a large-scale intragenic deletion. There was a male : female sex ratio of 0.89. The intragenic deletion, c.2295-866_2410-30del, was identified in 8 of 17 patients (47%), all caucasians, and occurred against the background of a common haplotype, suggesting a founder effect [3]. In this study, 23 out of the 25 heterozygous parents were asymptomatic. Two, who suffered from cramps or moderate myalgia, harboured two different point mutations. The healthy sister of one patient, who had an *LPIN1* intragenic deletion, reported exercise-induced muscle pain [3].

▪ Diagnostic Tests

Since more than 50% of patients with 'unexplained' myoglobinuria, after exclusion of primary FAO disorders, have been found to be harbouring *LPIN1* mutations, LPIN-1 deficiency should be regarded as a major cause of severe recurrent myoglobinuria in early childhood. The high frequency of the intragenic *LPIN1* deletion should provide a valuable criterion for fast diagnosis, prior to muscle biopsy. Given that an *LPIN1* mutation has been detected in an individual who developed severe statin-induced myopathy, screening of a larger number of statin-treated patients and controls may be warranted [2, 3] (▶ Chapter 32).

▪ Treatment and Prognosis

Treatment with intravenous fluids and alkalinisation are required to maintain normal plasma creatinine and urinary output during episodes. In the reported cases death has occurred in a number of patients during episodes.

35.1.2 Cardiolipin Remodelling Enzyme Deficiency: Barth Syndrome

▪ Clinical Presentation

The classic presentation of Barth syndrome, an X-linked recessive disorder, includes cardiomyopathy, skeletal muscle weakness, neutropenia and growth retardation [8]. Elevated urinary excretion of 3-methylglutaconic acid and hypocholesterolaemia are typically present [9]. There is, however, considerable variability in the age of onset, the expression of symptoms and the progression of the disease. At its most severe the disorder can cause increased male fetal loss, stillbirth [10] or neonatal death, but by contrast, a mild cognitive phenotype has also been described. The current patient population covers a wide range of individuals, from those who have severe debilitating disease to those who are nearly asymptomatic. The majority of patients registered with the Barth Syndrome Foundation (www.barthsyndrome.org) are children. Cardiomyopathy is the most serious manifestation and presents either as biventricular dilatation or as left-ventricular noncompaction [11]. Sudden episodes of cardiac deterioration are common and are often followed by unexplained remissions.

▪ Metabolic Derangement

Barth syndrome is a mitochondrial disorder caused by abnormalities in cardiolipin (CL). CL is an important phospholipid of the mitochondrial membrane of eukaryotes. It is primarily found in the inner mitochondrial membrane, and to a lesser extent also in the outer mitochondrial membrane [12]. CL is synthesised from its precursors,

phosphatidylglycerol and cytidinediphosphate-diacylglycerol, and after primary synthesis CL acyl chains are remodelled to achieve their final mature composition. To exert its function properly, it is essential that CL is present in a symmetrical mature form; the *Tafazzin* (*TAZ*) gene encodes a CL-remodelling enzyme, which maintains the symmetry of acyl chains in the cardiolipin structure (step 14 in ◘ Fig. 35.1) [13]. The *TAZ* gene product has recently been identified as 59-kDa protein named monolysocardiolipin acyltransferase-1 (MLCL AT-1) [14].

Deficiency of CL results in mitochondria that contain reduced levels of cardiolipin, and the remaining cardiolipin lacks its characteristic acyl pattern. The exact consequences for mitochondrial function remain to be established, but may include deficiencies in mitochondrial energy coupling and/or in mitochondrial biogenesis. Mitochondrial abnormalities in Barth syndrome compromise the development and function of certain tissues, such as skeletal and heart muscle, in which high energy requirements necessitate precise structural organisation of mitochondria. Leakage from mitochondria of metabolites from leucine catabolism may be a cause of the methylglutaconic aciduria.

▪ Genetics

The *TAZ* gene is located in the gene-rich region Xq28. Different mRNAs were produced by alternative splicing of the primary *TAZ* transcript, encoding proteins that differed at their N-terminus and in the central region. Mutations have been identified in exons 2, 3 and 7 and in the 3-prime splice junction of intron 2, including splice site mutations, deletions, insertions, missense mutations and nonsense mutations [15, 16]. Review of the *TAZ* mutations identified to date in 38 reported cases of Barth syndrome and other cardiomyopathies has revealed no correlation between location or type of mutation and either cardiac phenotype or disease severity [16].

▪ Diagnostic Tests

Urine organic acid analysis by GCMS, which may be used as an initial screening test, will identify the presence of 3-methyl glutaconic and 3-methyl glutaric acids. However, although this abnormal excretion can be observed at any age it is not constant [17]. Barth syndrome is classified as 3-methyl glutaconic aciduria type II with a normal 3-methyl glutaconyl-CoA hydratase activity (▶ Chapter 19). Cells from affected patients, including lymphocytes, fibroblasts and muscle, show an increased monolysocardiolipin : cardiolipin ratio (MLCL/CL) [18]. A rapid bloodspot screening method based on the measurement of this ratio by tandem mass spectrometry analysis is now available and appears to be both specific and sensitive [19]. Diagnosis is confirmed by molecular analysis of the *TAZ* gene.

▪ Treatment and Prognosis

No specific treatment is available. Prognosis is highly variable and depends primarily on the severity of cardiac involvement. Granulocyte colony-stimulating factor may be necessary for treatment of neutropenia. Cardiomyopathy may respond to standard therapy, but some patients have required heart transplantation [20].

35.1.3 Phospholipase A2 Deficiency (Infantile Neuroaxonal Dystrophy and Neurodegeneration with Brain Iron Accumulation)

▪ Clinical Presentation

Phospholipase A2 deficiency (encoded by the *PLA2G6* gene) is responsible for infantile neuroaxonal dystrophy (INAD) and for neurodegeneration associated with brain iron accumulation (NBIA). INAD and NBIA share distinctive pathological features of neuroaxonal degeneration with distended axons (spheroid bodies) throughout the CNS. These clinical entities have been also collectively termed *PLA2G6*-associated neurodegeneration (PLAN). Patients with PLAN usually present within the first 2 years of life, and the disease culminates in death by the age of 10 years.

Patients with INAD show a progressive disorder with motor and mental deterioration, cerebellar ataxia, marked hypotonia of the trunk with later bilateral pyramidal tract signs, spastic tetraplegia, hyperreflexia and early visual disturbances. Seizures are not reported, but electroencephalography shows characteristic high-voltage fast rhythms; electromyography results are consistent with chronic denervation. Most patients have abnormal visual evoked potentials.T_2-weighted magnetic resonance imaging (MRI) typically shows cerebellar atrophy with signal hyperintensity in the cerebellar cortex and, occasionally, hypointensity in the pallida and substantia nigra corresponding to iron deposits. Pathological hallmarks are marked neuroaxonal dystrophy, severe cerebellar atrophy and degeneration of the lateral corticospinal tracts. Axonal endings show spheroid bodies, often detectable in the skin and conjunctivae [21, 22].

Patients with NBIA may exhibit a milder phenotype than patients with INAD, with static encephalopathy in childhood and late-onset neurodegeneration in adolescence or adulthood. In addition, *PLA2G6* was also reported recently as the causative gene for PARK14, a form of autosomal recessive early-onset dystonia-Par-

kinsonism. Patients with this clinical form display L-dopa-responsive dystonia/Parkinsonism starting in the 2nd or 3rd decade of life, together with pyramidal signs, cognitive decline and psychiatric disorders. Brain MRI typically shows mild generalised cerebral atrophy on MRI but no iron accumulation [23, 24].

The NBIA entity now encompasses, besides *PLA2G6*-associated neurodegeneration, two other disorders, fatty acid hydroxylase deficiency (*FADH2* mutations) and pantothenate kinase-associated neurodegeneration (*PANK2* mutations), that show overlapping clinical features but distinctive radiological abnormalities (► below and ► Chapter 2). Another disease to be considered in the differential diagnosis of INAD is Schindler disease type I, which is caused by mutations in α-*N*-acetylgalactosaminidase (NAGA) (► Chapter 38).

▪ Metabolic Derangement

The *PLA2G6* gene encodes phospholipase A2 (PLA2), which is also known as iPLA2β (cytosolic, group VI, calcium independent). Alternative splicing results is a number of different PLA2s, the function of which is to catalyse the release of free fatty acids from phospholipids (step 7 in ◘ Fig. 35.1). They display, therefore, a number of physiological functions, including phospholipid remodeling, arachidonic acid release, leukotriene and prostaglandin synthesis, Fas-mediated apoptosis and transmembrane ion flux in glucose-stimulated beta cells [25]. Mutations in the *PLA2G6* gene causing a loss of enzyme activity may lead to alterations in cell signalling and membrane remodelling, with a subsequent accumulation of membranes, organelles and proteins that represent spheroids [26]. Recent studies indicate that different alterations in *PLA2G6* function produce the different disease phenotypes of NBIA/INAD and dystonia-Parkinsonism. It is suggested that INAD/NBIA is caused by loss of the ability of *PLA2G6* to catalyse fatty acid release from phospholipids, which predicts accumulation of *PLA2G6* phospholipid substrates and provides a mechanistic explanation for the accumulation of membranes in neuroaxonal spheroids. In contrast, mutations in dystonia-Parkinsonism do not appear to directly impair catalytic function, but may modify substrate preferences or regulatory mechanisms for *PLA2G6* [26].

▪ Genetics

PLA2G6 deficiency is an autosomal recessive trait caused by mutations in the *PLA2G6* gene. The human gene is located on chromosome 22q13.1, contains 19 exons and spans more than 69 kb. In studies of 12 families with infantile neuroaxonal dystrophy and a large consanguineous Pakistani family with neurodegeneration with brain iron accumulation, Morgan et al. [27], and Khateeb et al. [28] independently identified 44 unique mutations (32 missense, 5 deletions leading to frameshift, 3 nonsense, 2 leading to amino acid deletions without frameshift, 1 splice site, and 1 large deletion). Some specific mutations have been reported to be responsible for the L-dopa-responsive dystonia/Parkinsonism phenotype [23, 26, 27].

▪ Diagnostic Tests

On the basis of genetic findings which demonstrated *PLA2G6* mutations in INAD [28] molecular diagnosis of this disorder can, at least in part, replace the invasive biopsies used in the diagnosis and also allow for carrier detection, prenatal diagnosis and presymptomatic diagnosis in affected families.

▪ Treatment and Prognosis

No treatment is available.

35.1.4 α-/β-Hydrolase 12 (ABHD12) Deficiency (Polyneuropathy, Hearing Loss, Ataxia, Retinitis Pigmentosa and Cataracts: PHARC Syndrome)

▪ Clinical Presentation

Eighteen patients from 11 different families have been described so far [29, 30]. PHARC, both in the Norwegian patients and in the single American patient, appears to be a slowly progressive disease with recognition of the first symptoms typically in the teens. Cataracts, hearing loss and a predominantly demyelinating peripheral neuropathy are present in all adult patients, whereas the presence and extent of ataxia is variable. Extensor plantar response in the lower limbs is found in almost all patients. Retinitis pigmentosa typically presents in young adult life (twenties or thirties), and in most patients electroretinograms show a rod-cone dysfunction.

The disorder in families from Algeria and the Emirates shows an earlier onset of ataxia that has both central and peripheral characteristics. No evidence of behavioural disturbances has been detected in adult patients. Cerebral cortical function appears to be spared, with only one patient having mental retardation and one other myoclonic seizures. Most patients present a cerebellar atrophy on MR/CT of brain.

▪ Metabolic Derangement

PHARC syndrome has been recently linked to ABDH12 deficiency (step 10 in ◘ Fig. 35.1). ABDH12, with ABDH6 and monoacylglycerol lipase, is involved in hydrolysis of the endocannabinoid 2-arachidonoyl glycerol (2-AG) to arachidonic acid [30]. Although monoacylglycerol lipase

(MAGL) is responsible for 85% of 2-AG hydrolysis in mouse brain, ABHD12 and ABHD6 may be important for hydrolysis in specific cell types and/or cellular compartments. 2-AG and *N*-arachidonoylethanolamine (anandamide) are endogenous ligands of cannabinoid receptors CB1 and CB2 and are reported to be involved in a broad range of physiological functions, such as emotion, energy metabolism regulation, reproduction and synaptic plasticity. This serious and progressive disease suggests that ABHD12 performs an essential function in the peripheral and central nervous systems and in the eye. This is supported by the high expression of ABHD12 in the brain, with striking enrichment in microglia. Currently, the only known substrate for ABHD12 is the main endocannabinoid 2-arachidonoyl glycerol (2-AG). 2-AG is also a substrate for the inducible enzyme cyclooxygenase-2 (COX2), which is involved in neuroinflammation. COX2 converts 2-AG to the corresponding hydroperoxy derivative, which is further metabolised to prostaglandin E2 glycerol ester by prostaglandin E2 glycerol ester synthase (PGE2S). The apparent paradox of a purported minor role of ABHD12 in 2-AG hydrolysis versus the serious PHARC phenotype in the brain and eye suggests either that ABHD12 is of crucial importance only in certain cell types or that it is also acting on a hitherto unknown substrate other than 2-AG [30].

- **Genetics**

The *ABHD12* gene maps to chromosome 20 [29]. Four mutations affecting 18 patients from 11 different families have been described so far, namely Norway mutation: c.337_338delGAinsTTT (8 patients from 5 families); Emirates mutation: 14-kb deletion removing exon 1 (3 patients from 1 family); Algeria mutation: c.846_852dupTAAGAGC (7 patients from 4 families); and finally, a patient from the USA of French-Canadian heritage with suspected PHARC disease was found to be homozygous for a nonsense mutation (c.1054C>T) in exon 12 [30]. Each of the four different *ABHD12* mutations is interpreted as a null mutation that would either abolish or severely reduce the activity of the encoded enzyme, α-/β-hydrolase12 (ABHD12).

- **Diagnostic Tests**

While the clinical picture is reminiscent of Refsum disease, affected individuals have normal phytanic and pristanic acid levels in plasma, as well as normal enzymatic activity for alpha oxidation and other peroxysomal functions. The combination of retinitis pigmentosa and ataxia can lead to checks for spinocerebellar ataxia type 7 (SCA7) and sequencing of mitochondrial DNA for the mutations that cause neuropathy, ataxia and retinitis pigmentosa (NARP) [29]. No biochemical marker is available so far, and diagnosis relies entirely on molecular analysis [30].

- **Treatment and Prognosis**

There is no treatment available.

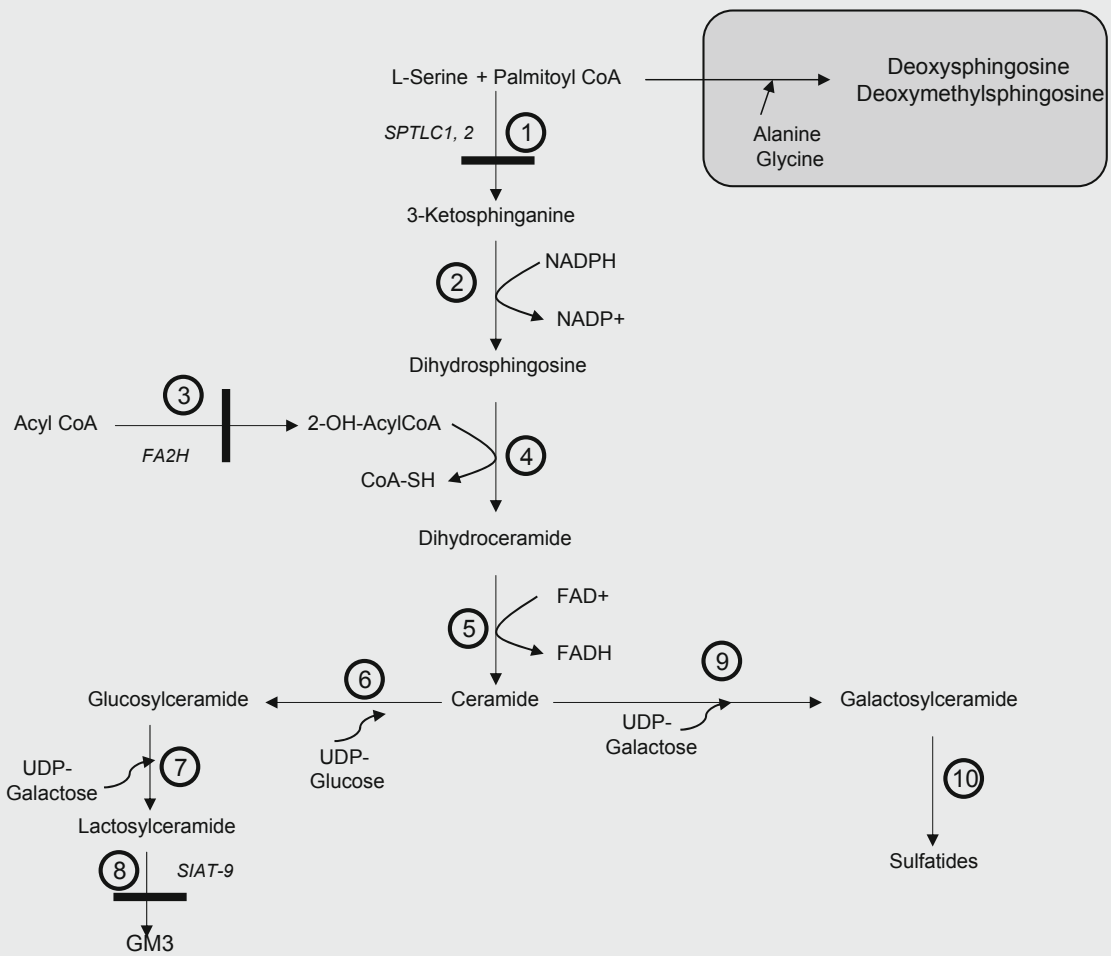
35.1.5 Choline Kinase Deficiency (Congenital Muscular Dystrophy with Mitochondrial Structural Abnormalities)

Homozygotes or compound heterozygotes mutations in the gene encoding choline kinase beta have been recently described in 15 individuals with a congenital muscular dystrophy characterized by early-onset muscle wasting, mental retardation and peculiar enlarged mitochondria [31].

Glycosphingolipid Biosynthesis

Glycosphingolipids are constituents of cell membranes, particularly in neuronal cells. They are distinguished from glycolipids by the fact that the polar head groups consist of one or more sugar residues instead of phosphate groups. Synthesis of glycosphingolipids begins with the formation, by L-serine palmitoyltransferase, of 3-ketosphinganine (also named 3-ketodihydrosphingosine). Reduction of this compound by 3-ketosphinganine reductase results in the formation of dihydrosphingosine (sphinganine). The latter is acylated by dihydroceramide synthase, which utilises 2-OH-acyl CoA produced by fatty acid 2-hydroxylase. Dihydroceramide is converted into ceramide by dihydroceramide desaturase.

Ceramide is the precursor of gangliosides, complex, sialic acid-containing molecules which play important structural and functional roles. Among them is GM3, which is formed via ceramide glucosyltransferase, lactosylceramide synthase and GM3 synthase. Ceramide is also the precursor of sulfatides, which are an important myelin constituent and mediate diverse biological processes. Their synthesis proceeds via galactosylceramide synthase and sulfogalactosyltransferase (◘ Fig. 35.2).



◘ **Fig. 35.2.** Major reactions involved in glycosphingolipids biosynthesis: *CoA*, coenzyme A; *UDP*, uridine diphosphate glucose. **1**, Serine palmitoyltransferase; **2**, 3-ketosphinganine reductase; **3**, fatty acid 2-hydroxylase; **4**, dihydroceramide synthase; **5**, dihydroceramide disaturase; **6**, ceramide glucosyltransferase; **7**, lactosylceramide synthase; **8**, GM3 synthase (sialyltransferase-9); **9**, galactosylceramide synthase; **10**, sulfogalactosyltransferase. Enzyme deficiencies are indicated by *solid bars* across the *arrows* The reaction catalysed by mutated *SPTLC1*, 2 which uses alanine or glycine rather than its natural substrate serine, leading to the formation and accumulation of the neurotoxic metabolites 1-deoxysphingosine and 1-deoxymethylsphingosine, is shown in the *box*

35.2 Disorders of Glycosphingolipid Synthesis

Serine palmitoyltransferase (SPT) deficiency causes the hereditary sensory autonomic neuropathy type I (HSAN I) characterised by prominent predominantly distal sensory loss, autonomic disturbances and motor neuron degeneration of adult onset. It is transmitted as an autosomal dominant trait.

Fatty acid 2-hydroxylase deficiency causes a subtype of neurodegeneration with brain iron accumulation with a later age of onset and slower progression.

GM3 synthase deficiency has been described in a unique Amish family presenting with an autosomal recessive infantile severe epilepsy syndrome associated with developmental arrest and blindness.

35.2.1 Serine Palmitoyl CoA Transferase Deficiency

▪ Clinical Presentation

Serine palmitoyl CoA transferase deficiency has recently been identified as a cause of hereditary sensory autonomic neuropathy type I (HSAN I), a slowly progressive neurological disorder characterised by prominent, predominantly distal sensory loss, autonomic disturbances, autosomal dominant inheritance and juvenile or adult disease onset. Disease onset varies between the 2nd and 5th decades of life. The main clinical feature of HSAN I is the reduction of sensation, which is mainly distributed to the distal parts of the upper and lower limbs. Variable distal muscle weakness and wasting, and chronic skin ulcers are characteristic. Autonomic symptoms, characterised by loss of pain and temperature sensation in the feet and hands, often accompanied by attacks of shooting pain and sweating, occur frequently [31]. In addition, motor neuron degeneration may occur and is responsible for atrophy and weakness of distal limb muscles. Serious and common complications are spontaneous fractures, osteomyelitis and necrosis, and also neuropathic arthropathy, which may necessitate amputations. Hypoacousis or deafness, cough and gastro-oesophageal reflux have been observed in rare cases. Diagnosis is based on the clinical findings and is supported by a family history. Nerve conduction studies confirm a sensory and motor neuropathy predominantly affecting the lower limbs.

▪ Metabolic Derangement

The *SPTLC1* and *-2* genes code for serine palmitoyltransferase (SPT), a dimer consisting of an SPTLC1 subunit associated with either SPTLC2 or SPTLC3 subunits [32]. SPT catalyses the pyridoxal-5'-phosphate-dependent condensation of serine with palmitoylCoA. This is the first and rate-limiting step in sphingolipid biosynthesis (step 1 in ◘ Fig. 35.2). Sphingolipids serve not only as components of biological structures such as membranes and lipoproteins, but also as regulators of cell proliferation, differentiation, cell-cell and cell-matrix interaction, cell signalling and membrane trafficking. Recently, it has been demonstrated that the common mutations in the active site of SPT causing HSAN1 induce a shift in its substrate specificity [33]. Alanine and glycine, rather than serine, are used by mutant SPT, leading to the formation of atypical deoxysphingoid bases (DSBs). Owing to the lack of hydroxyl group on C1, these atypical compounds can neither be converted to complex sphingolipids nor be degraded (◘ Fig. 35.2). DSBs are neurotoxic and interfere with the formation of neurites in vitro.

▪ Genetics

HSAN1 linked to *SPTLC* mutations is an autosomal dominant disease. The *SPTLC1* gene is located on chromosome 9q22.1-q22.3 and contains 15 exons, and the *SPTLC2* gene is located on chromosome 14q24.3. Dawkins et al. [34] and Bejaoui [35] independently identified mutations in *SPTLC1* in about 15 families with HSAN I. These mutations have a dominant-negative effect on the SPT enzyme. The G387A *mutation* was described in one family and seems not to be disease causing. Recently, two heterozygous mutations affecting the *SPLC2* gene and also associated with HSAN1 were reported, and a third one, 1510A>T, was reported as a de novo mutation with an atypical early-onset HSAN1 [32].

▪ Diagnostic Tests

The recently reported elevated DSBs levels in plasma from HSAN1 patients, which can be measured by tandem mass spectrometry, provide a specific and noninvasive diagnostic test, which can then be confirmed by molecular analysis of the *SPTLC1* and *C2* genes [32, 33].

▪ Treatment and Prognosis

Mutilating ulcerative complications are the most serious and prominent signs and the leading diagnostic feature in HSAN I. The painless neuropathic foot ulcerations observed in several subtypes of autosomal dominant HSAN I often mimic foot ulcers caused by diabetic neuropathy and thus resemble a '*pseudodiabetic foot syndrome*'. The guidelines given for diabetic foot care can be also used in HSANI. If patients with HSANI receive appropriate counselling and treatment their prognosis is good. The disease is slowly progressive and does not influence life expectancy [31].

35.2.2 Fatty Acid 2-Hydroxylase Deficiency

Clinical Presentation

The clinical phenotypes associated with fatty acid 3-hydroxylase (*FA2H*) mutations, previously known to cause leukodystrophy and a form of hereditary spastic paraplegia (SPG35) [36, 37], have recently been classified as a subtype of neurodegeneration with brain iron deposition (NBIA, see above). Onset is usually in childhood with slow progression, but can also occur in adulthood. Affected patients demonstrate a profound ataxia, and dysmetria with mild to severe dystonia. Spastic quadriplegia with pyramidal tract signs is prominent, with near-normal intellect [38]. Retinopathy or optic atrophy with nystagmus and acquired strabismus are present in all affected patients and may be diagnostic clues. The lack of peripheral neuropathy may be related to the presence of a second fatty acid hydroxylase activity in peripheral tissues. Imaging findings with MRI demonstrate bilateral T2 hypointensity in the globus pallidus, consistent with the iron deposition characteristic of NBIA, with periventricular white matter hyperintensity and thinning of the corpus callosum. A profound cerebellar atrophy is evident, in addition to mild generalised cortical atrophy [38].

Metabolic Derangement

FA2H is highly expressed in primarily oligodendrocytes in brain and produces 2-hydroxylated acylCoA, which is required for de novo synthesis of dihydroceramide and ceramide (step 3 in ◘ Fig. 35.2). A direct interaction of FA2H with iron-containing moieties, as suggested by a predicted sequence homology, may explain the iron accumulation observed in FA2H deficiency [36, 39]. In addition to the role of FA2H in ceramide production, mutations could affect intracellular ceramide pool composition, leading to premature apoptosis. Macrophage PAS-positive granular cytoplasm can be observed in bone marrow biopsy specimens, which is thought to indicate a lysosomal storage disease [39].

Genetics

FA2H deficiency is a recessive autosomal disease linked to mutations in the *FA2H* gene located on chromosome 16q23. The gene contains seven exons and codes for a 372-amino acid (42.8-kDa) FA2H protein. The first mutation, 786+1G>A, was identified in DNA from seven patients from two unrelated consanguineous families. The mutation is associated with leukodystrophy, spastic paraparesis and dystonia. A second mutation, 103G>T, was identified in a third family with a less severe phenotype [36]. More recently two other homozygous mutations were identified in two unrelated families [39].

Diagnostic Tests

The clinical diagnosis of FA2H deficiency is based on the observation of signs and symptoms similar to those observed in NBIA, but with a later age of onset and slower progression, and supported by a family history. Diagnosis is confirmed by molecular analysis of the *FA2H* gene.

Treatment and Prognosis

There is no specific treatment available.

35.2.3 GM3 Synthase Deficiency

A disorder caused by loss of function mutation in the gene for GM3 synthase, also called lactosylceramide-α-2,3 sialyltransferase (SIAT-9), was the first inherited metabolic disease linked to a defect in complex glycolipids biosynthesis [40]. GM3 synthase is a member of the sialyltransferase family and catalyses the initial step in the biosynthesis of most complex gangliosides (step 8 in ◘ Fig. 35.2). Plasma from affected individuals exhibited a lack of GM3 ganglioside and its biosynthetic derivatives.

The mutation causes an autosomal recessive infantile severe epilepsy syndrome associated with developmental stagnation and blindness. Seizure activity reported to be difficult to control started within the 1st year of life. This mutation was identified on chromosome 9 in a large Amish pedigree. So far it has been reported in only one publication. This apparent rarity might be due to the poor clinical description of the clinical phenotype and the rather complicated method for measuring GM3 in plasma.

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VIII Disorders of Nucleic Acid and Heme Metabolism

Disorders of Purine and Pyrimidine Metabolism

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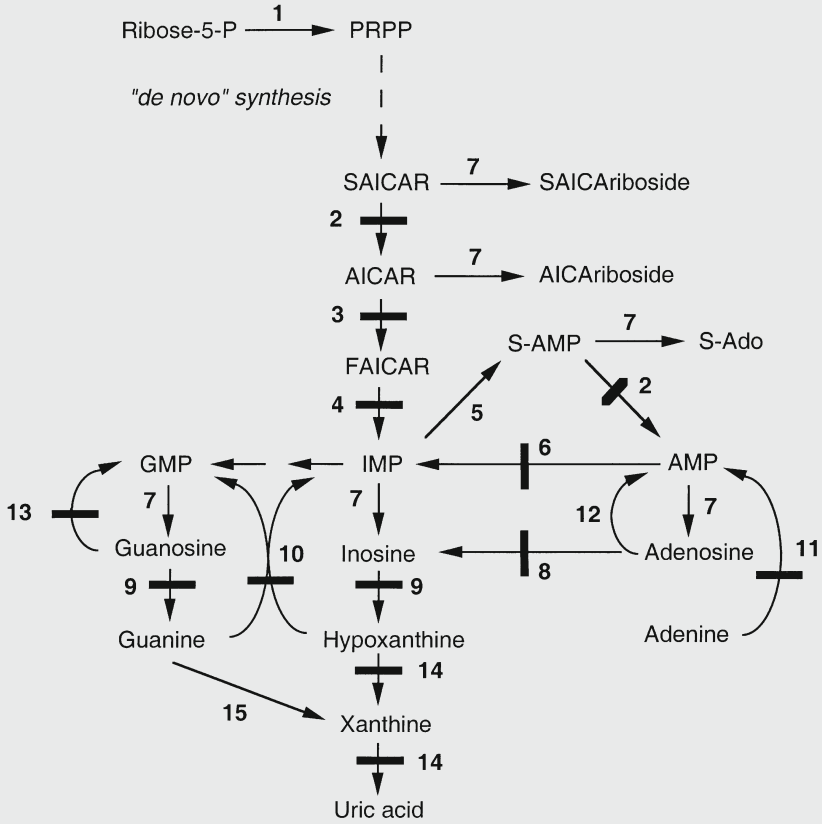
Purine Metabolism

Purine nucleotides are essential cellular constituents, which intervene in energy transfer, metabolic regulation and synthesis of DNA and RNA. Purine metabolism can be divided into three pathways (◘ Fig. 36.1):

- 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 crystallise once its plasma concentration surpasses 6.5-7 mg/dl (0.38-0.47 mmol/l).
- The salvage pathway utilises the purine bases, guanine, hypoxanthine and adenine, which are provided by food intake or the catabolic pathway, and reconverts them to, respectively, GMP, IMP and AMP. Salvage of the purine nucleosides, adenosine and guanosine, and their deoxy counterparts, catalysed by kinases, also occurs. The salvage pathway also converts several pharmacological anticancer and antiviral nucleoside analogues into their active forms.



◘ **Fig. 36.1.** 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); **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, which are depicted in ◘ Figs. 36.1, 36.2 and 36.3, respectively.

36.1 Inborn Errors of Purine Metabolism

Inborn errors of purine metabolism comprise defects of:

- **Purine nucleotide synthesis:** phosphoribosylpyrophosphate (PRPP) synthetase superactivity and deficiency, adenylosuccinase (ADSL) deficiency, AICA-ribosiduria caused by ATIC deficiency;
- **Purine catabolism and interconversions:** the deficiencies of muscle AMP deaminase (AMP-DA, also termed myoadenylate deaminase), adenylate kinase 2, adenosine deaminase (ADA), purine nucleoside phosphorylase (PNP) and xanthine oxidase;
- **Purine salvage:** the deficiencies of hypoxanthine-guanine phosphoribosyltransferase (HPRT) and adenine phosphoribosyltransferase (APRT). The deficiency of deoxyguanosine kinase causes mitochondrial DNA depletion (see also ► Chapter 15).

Deficiency of thiopurine *S*-methyltransferase (TPMT, not shown in ◘ Fig. 36.1) results in less efficient methylation and hence in enhanced toxicity of pharmacological thiopurine analogues. Deficiency of inosine triphosphate pyrophosphatase (ITPase, not shown in ◘ Fig. 36.1) also increases the toxicity of thiopurines. With the exception of the deficiencies of muscle AMP-DA and TPMT, all these enzyme defects are very rare.

36.1.1 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 [1, 2]. Uricaemia can be very high, reaching 10-15 mg/dl (0.60-0.90 mmol/l; normal adult values: 2.9-5.5 mg/dl [0.17-0.32 mmol/]). The urinary excretion of uric acid is also increased, reaching up to 2,400 mg (14 mmol)/24 h, or 2.5 mmol/mmol creatinine (normal adult values: 500-800 mg [3-4.7 mmol])/24 h, or 0.2-0.3 mmol/mmol creatinine.

A few patients have been reported in whom clinical signs of uric acid overproduction appeared even in infancy and were accompanied by neurological abnormalities, mainly sensorineural deafness, particularly for high tones, but also hypotonia, locomotor delay, ataxia and autistic features [2].

▪ Metabolic Derangement

The enzyme forms phosphoribosyl pyrophosphate (PRPP) from ribose-5-phosphate and ATP (◘ Fig. 36.1). PRPP is the first intermediate of the de novo synthesis of purine nucleotides. PRPP synthetase is highly regulated. Various genetic regulatory and catalytic defects [3] lead to superactivity, resulting in increased generation of PRPP. Because PRPP amidotransferase, the 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. The mechanism of the neurological symptoms is unresolved.

▪ 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 [2]. Genetic studies have revealed that PRPP synthetase superactivity is caused either by distinctive gain-of-function point mutations in the *PRPS1* gene (in the severe phenotype), or by acceleration of the transcription of a normal *PRPS1* isoform (in the milder phenotype) [3, 4].

▪ Diagnostic Tests

Diagnosis requires extensive kinetic studies of the enzyme, which are performed on erythrocytes and cultured fibroblasts in a few laboratories in the world. The disorder should be differentiated from partial HPRT deficiency, which gives similar clinical signs.

▪ Treatment and Prognosis

Patients should be treated with allopurinol, which inhibits xanthine oxidase, the last enzyme of purine catabolism (◘ Fig. 36.1). This results in a decrease in the production of uric acid and in its replacement by hypoxanthine, which is about 10 times as 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 [5]. Consequently, additional measures to prevent cristallisation 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 uricaemia prevents gouty arthritis and urate nephropathy, but does not correct the neurological symptoms.

36.1.2 Phosphoribosyl Pyrophosphate Synthetase Deficiency

In recent years, several loss-of-function mutations of the *PRPS1* gene have been identified in a number of patients with X-linked hearing impairment, including Arts syndrome, Charcot-Marie Tooth disease 5, and X-linked nonsyndromic hearing loss [4]. PRPP synthetase activity is decreased, although not completely deficient. There is no clear reduction of serum and urinary uric acid, suggesting that the defect could be compensated.

36.1.3 Adenylosuccinase (Adenylosuccinate Lyase) Deficiency

▪ Clinical Picture

The clinical spectrum of the deficiency of adenylosuccinate lyase (ADSL, also called adenylosuccinase) is very broad, ranging from fatal neonatal convulsions to mild psychomotor retardation, muscle hypotonia and autistic behaviour. About 60 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 [6, 7]. Other patients present with intractable convulsions starting within the first days to weeks of life [8], often with severe muscular hypotonia necessitating mechanical ventilation [9], and lead to death or severe mental retardation. Impaired intrauterine growth, microcephaly, fetal hypokinesia, and a lack of fetal heart rate variability have also been observed. Rare patients, referred to as type II, are only mildly retarded [7]. One boy displayed profound muscle hypotonia accompanied by slightly delayed motor development [10]. A few others display mainly autistic behaviour [11]. Recently, the deficiency was diagnosed in two sisters with a behavioural phenotype resembling Angelman syndrome [12]. Microcephaly is often present. Computed tomography and magnetic resonance imaging of the brain show brain atrophy and hypomyelination, with hypotrophy or hypoplasia of the cerebellum, particularly of the vermis [13]. The marked clinical heterogeneity justifies systematic screening for the deficiency in both profound and mild unexplained psychomotor retardation, in neurological disease with convulsions and/or hypotonia and in individuals with autistic behaviour.

▪ Metabolic Derangement

ADSL catalyses two steps in purine synthesis (◘ Fig. 36.1): the conversion of succinylaminoimidazole carboxamide ribotide (SAICAR) to AICAR, along the de novo pathway, and that of adenylosuccinate (S-AMP) to AMP. Its deficiency results in accumulation in cerebrospinal fluid (CSF) and urine of the succinylpurines, SAICA riboside and succinyladenosine (S-Ado), the products of the dephosphorylation, by 5'-nucleotidase(s), of the two substrates of the enzyme. Present evidence indicates that the more severe presentations of ADSL deficiency tend to be associated with S-Ado/SAICA riboside ratios of around 1, whereas in milder clinical pictures these ratios are between 2 and 4. It suggests that SAICA riboside is the offending compound, and that S-Ado could protect against its toxic effects. The ADSL defect is marked in liver and kidney and is variably expressed in erythrocytes, muscle and fibroblasts [6, 7, 14]. The higher S-Ado/SAICA riboside ratios might be explained by a more profound loss of activity of the enzyme toward S-AMP than toward SAICAR, as compared with a parallel deficiency in severely affected patients [14]. The symptoms of the deficiency remain unexplained.

▪ Genetics

The deficiency is transmitted as an autosomal recessive trait [6, 7]. Studies of the *ADSL* gene, which is localised on chromosome 22, have led to the identification of about 50 mutations [15-17] (ADSL mutations database home page, http://www.icp.ucl.ac.be/adsldb/). Most are missense mutations, but a splicing error [16] and a mutation in the 5'UTR [18] have also been identified. Most frequently encountered, particularly in The Netherlands, and accounting for about one-third of the alleles investigated, is an R462H mutation.

▪ Diagnostic Tests

Diagnosis is based on the presence in CSF and urine of SAICA riboside and S-Ado, which are normally undetectable. For systematic screening, a modified Bratton-Marshall test [19] performed on urine appears most practical. False-positive results are, however, recorded in patients who receive sulphonamides. Final diagnosis requires HPLC with UV detection [6] or LCMS-MS [20, 21]. The activity of ADSL can be measured in RBC. It is deficient in some patients [14] but not in others [6]. Pre-

natal 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) 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 [7]. Oral administration of ribose (10 mmol/kg per day) [23] was also found to have no effect.

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.

36.1.4 AICA-Ribosiduria (ATIC Deficiency)

In a single patient [24] with profound mental retardation, marked dysmorphic features and congenital blindness, 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. 36.1). Assay of ATIC, the bifunctional enzyme catalysing the two last steps of de novo purine biosynthesis, revealed a profound deficiency of AICAR transformylase and a partial deficiency of IMP cyclohydrolase. The discovery of this inborn error of purine synthesis reinforces the necessity to perform a Bratton-Marshall test [19] in all cases of unexplained mental retardation and/or neurological symptoms.

36.1.5 Muscle Adenosine Monophosphate Deaminase Deficiency

▪ Clinical Picture

The deficiency of muscle AMP deaminase (AMP-DA, 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 AMP-DA defect is termed primary, present with isolated muscular weakness, fatigue, cramps or myalgias following moderate to vigorous exercise, sometimes accompanied by an increase in serum creatine kinase and minor electromyographic abnormalities [25]. The relationship between clinical symptoms and the enzyme defect remains uncertain. Muscular wasting or histological abnormalities are absent. Secondary AMP-DA deficiency is found in association with several neuromuscular disorders, including amyotrophic lateral sclerosis, fascioscapulohumeral myopathy, Kugelberg-Welander syndrome, polyneuropathies and Werdnig-Hoffmann disease [26, 27].

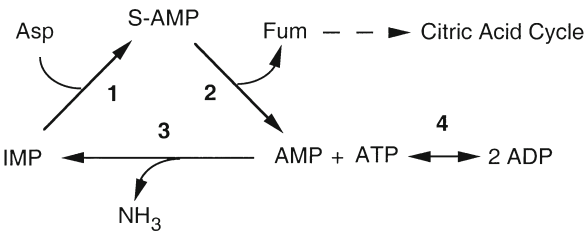
▪ Metabolic Derangement

AMP-DA, adenylosuccinate synthetase and adenylosuccinase form the purine nucleotide cycle (◘ Fig. 36.2). Numerous functions have been proposed for this cycle in muscle (reviewed in [28]), and it has been proposed that the muscle dysfunction observed in primary AMP-DA deficiency is caused by impairment of energy production for muscle contraction. However, this does not tally with the vast number of asymptomatic AMP-DA-deficient individuals, and suggests that the deficiency might have a synergistic effect in association with other disorders [29].

It should be noted that muscle, liver and erythrocytes contain different isoforms of AMP-DA. A regulatory mutation of liver AMP-DA has been proposed as a cause of primary gout with overproduction of uric acid [30]. Individuals with a complete but totally asymptomatic deficiency of erythrocyte AMP-DA have been detected in Japan, Korea and Taiwan [31].

▪ Genetics

Primary AMP-DA 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 an inactive enzyme. Population studies show that this mutant allele is found with a high frequency in caucasians. This accords with the finding that about 2% of diagnostic muscle biopsies are AMP-DA deficient. More recently, other,



◘ **Fig. 36.2.** 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)

rarer mutations of the *AMPD1* gene have been identified in AMP-DA deficient individuals. The high frequency of AMP-DA deficiency suggests that it may confer some selective advantage, such as reduced prevalence of the metabolic syndrome [33].

▪ Diagnostic Tests

Screening for the defect can be performed by an exercise test (► Chapter 4). A several-fold elevation of venous plasma ammonia, seen in normal subjects, is absent in AMP-DA deficiency. The final diagnosis is established by histochemical or biochemical assay in a muscle biopsy. In the primary defect, the activity of AMP-DA 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 [26, 27].

▪ Treatment and Prognosis

Patients may display a gradual progression of their symptoms, which may culminate in a state where even dressing and walking a few steps lead to fatigue and myalgias. They should be advised to exercise with caution to prevent rhabdomyolysis and myoglobinuria. Administration of ribose (2-60 g per day orally in divided doses) has been reported to improve muscular strength and endurance [35].

36.1.6 Adenylate Kinase 2 Deficiency

Adenylate kinase 2 (AK2) is a mitochondrial enzyme that interconverts AMP, ADP and ATP (◘ Fig. 36.2) and therefore plays a critical role in energy homeostasis. AK2 deficiency has been described in reticular dysgenesis [36, 37], an autosomal recessive form of severe combined immunodeficiency (SCID), which is characterised by the absence of granulocytes and near-complete deficiency of lymphocytes in peripheral blood. These are caused by an early differentiation arrest in the myeloid lineage and impaired lymphoid maturation, and result in the occurrence of very severe infections at an earlier age than usually observed in other forms of SCID. The neutropenia does not respond to granulocyte colony-stimulating factor (G-CSF). In 13 affected individuals, biallelic mutations in AK2 have been identified [36, 37]. These mutations result in absent or strongly decreased protein expression. The restoration of AK2 expression in the bone marrow cells of individuals with reticular dysgenesis overcomes the neutrophil differentiation arrest [37]. All patients also display bilateral sensorineural deafness, which can be explained by the specific expression of AK2 in the stria vascularis region of the inner ear [37]. The only available treatment is allogeneic haematopoietic stem cell transplantation (HSCT).

36.1.7 Adenosine Deaminase Deficiency

▪ Clinical Picture

The clinical spectrum of adenosine deaminase (ADA) deficiency is very broad, ranging from a profound impairment of both humoural and cellular immunity in infants, known as **severe combined immunodeficiency disease** (SCID), to delayed and less severe later onset in older children or adults, and even benign partial ADA deficiency in adults [38, 39]. Within the first weeks or months after birth, approximately 80-85% of patients display multiple recurrent opportunistic infections caused by a variety of organisms, which rapidly become life threatening. Infections are mainly localised in the skin and in the respiratory and gastrointestinal tracts. In the gastrointestinal tract 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 the patients display bone abnormalities, which become evident clinically as prominence of and radiologically as cupping and flaring of the costochondral rib junctions. In some affected children, cognitive, behavioural and neurological abnormalities are present, including below-average IQ, hyperactivity, attention deficits, spasticity, head lag, nystagmus, inability to focus and high-frequency sensorineural deafness. Hepatic dysfunction has also been reported [40].

Approximately 15-20% of ADA-deficient children display clinical symptoms after 6 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 antithyroid antibodies. Allergies and elevated serum IgE are often present.

The very rare individuals who survive undiagnosed into the 1st 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, which is normally nearly undetectable (◘ Fig. 36.1), deoxyadenosine (not shown in ◘ Fig. 36.1),

another substrate of ADA, and their derivatives, notably deoxyadenosine triphosphate (dATP). These compounds induce the premature death of lymphoid progenitor cells, thereby profoundly impairing the generation of T,- B- and NK-lymphocytes. ADA deficiency has broad consequences, because in addition it affects bone, brain, lung, liver and perhaps also epithelial functions to varying extents.

▪ Genetics

ADA deficiency is an autosomal recessive disorder. It accounts for about 40% of North American, and for about 10-20% of 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 [39]. Most patients are compound heterozygotes. Spontaneous in vivo reversion to normal of a mutation on one allele, as observed in tyrosinaemia type I (► Chapter 18), has been reported [41].

▪ 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 and hypogammaglobulinaemia 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 immunisation 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 RBC. In general, severity of disease correlates with the loss of ADA activity: children with neonatal onset of SCID display 0-1% residual activity; in individuals with later onset 1-5% of normal ADA activity is found [38, 39]. It should be noted that only about 10-20% of patients with the clinical and haematological picture of inherited SCID are ADA 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 RBC but normal immunocompetence. This benign condition, called partial ADA deficiency, is explained by the presence of residual ADA activity in their lymphocytes.

▪ Treatment and Prognosis

Before the advent of modern therapies, ADA-deficient SCID almost invariably led to death, usually within the 1st year of life, unless drastic steps were taken, such as rearing affected infants 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 it has been extensively discussed in recent years [39, 42].

▪▪ Bone Marrow Transplantion

This remains the first choice, provided a fully matched sibling or family donor is available, and gives an approximately 70% chance of complete immunological cure. The graft provides haematopoietic 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 it is recommended that these be avoided unless no other treatment is available.

Although immune recovery after fully matched transplantation is generally excellent, the prognosis for nonimmunological clinical signs is not as good. The cognitive, behavioural and neurological abnormalities tend to persist, irrespective of the type of transplantation.

▪▪ Enzyme Replacement Therapy

Treatment with polyethylene glycol-modified bovine ADA (PEG-ADA) has now been used in approximately 200 patients [43, 44]. 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 neutralising antibodies against the bovine enzyme, autoimmunity and the high cost of lifelong therapy. Available evidence indicates that treatment with PEG-ADA 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 ADA deficiency in whom the *ADA* gene was inserted into peripheral blood T-cells, which live for a few months [45]. Later on, the gene was inserted into haematopoietic stem cells, which in theory have an unlimited life span. Results, in combi-

nation with a low-intensity, nonmyeloablative conditioning regimen, have been favourable in terms of both toxicity and efficacy [46]. It remains to be determined whether gene therapy will prove superior to fully matched sibling or family bone marrow transplantation in preventing cognitive, behavioural and neurological abnormalities. It should be mentioned that gene therapy in X-linked, not ADA-deficient SCID, although highly effective, has resulted in lymphoproliferative disorders owing to the integrated retroviral vectors, leading to searches for safer gene therapy [47, 48].

36.1.8 Adenosine Deaminase Superactivity

A hereditary, approximately 50-fold elevation of red cell ADA has been shown to cause nonspherocytic haemolytic anaemia [49]. This 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 patients with Blackfan Diamond anaemia and some of the coorresponding probands [50].

36.1.9 Purine Nucleoside Phosphorylase Deficiency

▪ Clinical Picture

Recurrent infections are usually of later onset, starting from the end of the 1st year and extending up to 5-6 years of age, and they are initially less severe than in ADA deficiency [51]. 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 neurological 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 anaemia, but also idiopathic thrombocytopenic purpura and autoimmune neutropenia. The disorder is much less frequent than ADA deficiency, with about 50 patients reported.

▪ Metabolic Derangement

The deficiency provokes an accumulation in body fluids of the four substrates of the enzyme, which are normally nearly undetectable, namely guanosine, inosine (◘ Fig. 36.1) and their deoxy counterparts (not shown in ◘ Fig. 36.1), the last derived from DNA breakdown. Formation of uric acid is thus severely hampered. The profound impairment of cellular immunity, characterising PNP deficiency, has been compounded by the greater ability of T-cells than of 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 nonimmunological symptoms in its deficiency.

▪ Genetics

The deficiency is inherited in an autosomal recessive fashion. Studies of the PNP gene, located on chromosome 14, have revealed a number of molecular defects, among which a R234P mutation was most common [52].

▪ 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, uricaemia may be at the borderline of normal. The urinary excretion of uric acid is usually also markedly diminished. Other causes of hypouricaemia, such as xanthine oxidase deficiency (see below) and drug administration (acetylsalicylic acid, thiazide diuretics), should be ruled out. Enzymatic diagnosis of PNP deficiency is usually performed on RBC.

▪ Treatment and Prognosis

Most initially diagnosed patients have died, although at a later age than untreated ADA-deficient children, from overwhelming viral or bacterial infections. Treatments consisted of bone marrow transplantation and repeated transfusions of normal, irradiated erythrocytes [51]. Successful matched bone marrow transplantation has been reported [53] without effect on neurological symptoms in one case [54] and with improvement in another [55]. Enzyme and gene therapy might become available in the future.

36.1.10 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: (1) type I classic xanthinuria, caused by isolated XO deficiency; (2) type II classic xanthinuria, due to deficiency of both XO and aldehyde oxidase (AO); (3) combined deficiency of XO, AO and sulfite oxidase [56]. Type I

and type II xanthinuria can be completely asymptomatic, although in about one third of cases kidney stones are formed. Most often not visible on X-ray, they may appear at any age, and provoke haematuria, 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 sulfite oxidase deficiency (which is also found as an isolated defect [57]; see also ►Chapter 21) overrides that of XO deficiency. The symptoms include neonatal feeding difficulties and intractable seizures, myoclonus, increased or decreased muscle tone, eye lens dislocation and severe mental retardation.

▪ 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. 36.1). As a rule, plasma hypoxanthine is not elevated, or only minimally, owing to its efficient reutilisation 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 metabolise synthetic purine analogues, such as allopurinol.

The combined deficiency of XO, AO and sulfite oxidase is caused by failure to synthesise a **molybdenum cofactor** (MoCo), which is common to the three oxidases. MoCo is synthesised by a complex pathway that involves four steps [58]. Approximately two-thirds of the patients with MoCo deficiency, classified as type A, lack the capacity to convert GTP to cyclic pyranoptrin monophosphate (pCMP), the first intermediate of MoCo synthesis. MoCo deficiency results, in addition to xanthinuria, in accumulation of neurotoxic sulfite and sulfur-containing metabolites and in diminution of the production of inorganic sulfate. Type II xanthine oxidase is caused by a defect in MoCo sulfurase, an enzyme that adds a terminal inorganic sulfur, required for the activity of both XO and AO, to MoCo.

▪ Genetics

The inheritance of the three types of XO deficiency is autosomal recessive. Type I xanthinuria is caused by mutations of the *XO* gene [59]. Type II xanthinuria results from mutations of the human MoCo sulfurase (*HMCS*) gene [60]. In combined XO, AO and sulfite oxidase deficiency, more than 30 different mutations in three MoCo biosynthetic genes have been identified [61]. The majority of these (type A) have mutations of the *MOCS1* gene [62], a bicistronic gene encoding two proteins which catalyse the first step of MoCo biosynthesis, the conversion of GTP to pCMP. The remainder (type B) have mutations in the *MOCS2* gene, a bicistronic gene that encodes two proteins which catalyse the second step of MoCo synthesis. A single patient has been reported with mutation of the *GEPH* gene, which encodes the enzyme that catalyses the third step of MoCo synthesis.

▪ 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 [62]. In combined XO, AO and sulfite oxidase deficiency, 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 that normally contain appreciable amounts of XO. Sulfite oxidase 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. Until recently, the prognosis of combined XO, AO and sulfite oxidase deficiency was very poor. Therapeutic attempts with low-sulfur diets, the administration of sulfate and molybdenum [56] and trials of thiol-containing drugs intended to bind sulfite have been unsuccessful. However, in a patient with MoCo deficiency type A_who was_diagnosed at the age of 6 days, daily infusions of 80-320 μg/kg per day of cPMP started on day 36 resulted in near-normalisation of the biochemical markers of the disorder and dramatic clinical improvement [63].

36.1.11 Hypoxanthine-guanine Phosphoribosyltransferase Deficiency

▪ Clinical Picture

The clinical spectrum of this disorder is very wide and is determined by the residual activity of the enzyme. Patients with complete or near-complete deficiency of hypoxanthine-guanine phosphoribosyltransferase (HPRT, sometimes abbreviated HGPRT) display Lesch-Nyhan

syndrome [64]. Affected children generally appear normal during the first months of life. At 3-6 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 [65]. 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 behaviour, both physical and verbal, toward others, such as spitting and use of shocking vocabulary, are also characteristic. Speech is hampered by athetoid dysarthria. Whereas most patients have IQs around 60-70, some display normal intelligence. Approximately 50% of these patients have seizures. Sooner or later they form uric acid stones. Mothers of Lesch-Nyhan patients have reported the finding of orange crystals on their affected sons' diapers during the first few weeks after birth. Untreated, the uric acid nephrolithiasis progresses to obstructive uropathy and renal failure during the 1st decade of life. The latter clinical picture may also be observed in early infancy in exceptional cases.

Partial HPRT deficiency is found in rare patients with gout. Most of them are normal on neurological examination, but occasionally spasticity, dysarthria and a spinocerebellar syndrome are found [66, 67]. Whereas most patients with Lesch-Nyhan syndrome do not develop gouty arthritis, this finding is common in partial HPRT deficiency. It is noteworthy that several females have been reported with Lesch-Nyhan syndrome. In adults HPRT deficiency presents with isolated dystonia and mild cognitive or behavioural problems [67] (► Chapter 2).

■ 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 [65]. 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. [68]. 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 [69].

■ Genetics

Both 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 [70]. So far, over 250 mutations of the *HPRT* gene have been described, ranging from point mutations to extensive deletions resulting in suppression of enzyme synthesis [70, 71]. Molecular studies have led to precise prenatal diagnosis and efficient carrier testing of at-risk females [72]. In females, Lesch-Nyhan syndrome is due to nonrandom or skewed inactivation of the X-chromosome [70].

■ Diagnostic Tests

Patients excrete excessive amounts of uric acid, ranging from 25 to 140 mg (0.15-0.85 mmol)/kg of body weight per 24 h, as against 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-year-olds, 10-year-olds and adults, respectively [73]. Increased ratios are also found in other disorders with uric acid overproduction, such as PRPP synthetase superactivity, glycogenosis type I and lymphoproliferative diseases. The overproduction of uric acid is generally accompanied by an increase of serum urate, which may reach concentrations as high as 18 mg/dl (1 mmol/l). Occasionally, however, particularly before puberty, uricaemia may be in the normal or high normal range. Female carriers may have elevated uric acid excretion.

Patients with Lesch-Nyhan syndrome display nearly undetectable HPRT activity in RBC [74]. 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 [75].

■ Treatment and Prognosis

Allopurinol is indicated to prevent urate nephropathy. Allopurinol, however, even when given from birth or in combination with adenine has no effect on the neurological symptoms [76], which have so far been resistant to all therapeutics attempts. Patients should be made more comfortable by appropriate restraints, including elbow splints and 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 [77]. In a single patient, chronic stimulation of the globus pallidus led to disappearance of self-mutilation [78]. In others it was unsuccessful and even led to a death [70].

36.1.12 Adenine Phosphoribosyltransferase Deficiency

- **Clinical Picture**

The deficiency may become clinically manifest in childhood, even from birth [79], but can also remain silent for several decades [80]. Symptoms include urinary passage of gravel, small stones and crystals, frequently accompanied by abdominal colic, dysuria, haematuria and urinary tract infections. Some patients may even present with acute anuric renal failure [81]. The urinary precipitates are composed of 2,8-dihydroxyadenine; they are radiotranslucent and indistinguishable from uric acid stones by routine chemical testing.

- **Metabolic Derangement**

The deficiency results in suppression of the salvage of adenine (◘ Fig. 36.1) provided by food and by the polyamine pathway. Consequently, adenine is oxidised by xanthine oxidase into 2,8-dihydroxyadenine, a very poorly soluble compound (solubility in urine, at pH 5 and 37°C, is about 0.3 mg/dl, as against 15 mg/dl for uric acid).

The deficiency can be complete or partial. The partial deficiency is only found in the Japanese, among whom it is quite common [82].

- **Genetics**

APRT deficiency is inherited as an autosomal recessive trait. All 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 [80, 83].

- **Diagnostic Tests**

Identification of 2,8-dihydroxyadenine requires complex analyses, including UV and infrared spectrography, mass spectrometry and X-ray cristallography [79]. It is therefore usually easier to measure APRT activity in RBC.

- **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. Alkalinisation of the urine is not advised, however: unlike that of uric acid, the solubility of 2,8-dihydroxyadenine does not increase up to pH 9.

The ultimate prognosis depends on the renal function at the time of diagnosis. It should be noted that kidney transplantation has been reported to be followed by recurrence of microcrystalline deposits and subsequent loss of graft function [80, 84].

36.1.13 Deoxyguanosine Kinase Deficiency

Two forms of deoxyguanosine kinase (DGUOK) deficiency, identified in approximately 100 patients [85], are known. The majority have the hepatocerebral form, a multisystemic mitochondrial DNA depletion syndrome (see also ▶ Chapter 15), characterised within weeks of birth by cholestasis and progressive liver failure, neurological abnormalities (severe hypotonia, developmental regression, rotary nystagmus evolving into opsoclonus), hypoglycaemia 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 [85, 86]. This enzyme phosphorylates the deoxycounterpart of guanosine (◘ Fig. 36.1) to deoxyGMP 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 [86]. Since then, other mutations have been identified. Both the hepatocerebral and the isolated hepatic disease have been observed in families harbouring the same mutations [85].

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 [87]. Administration of deoxynucleotides might become a therapeutic option in view of its favourable effect in vitro [88].

36.1.14 Thiopurine Methyltransferase Deficiency

Thiopurine *S*-methyltransferase (TPMT, not shown in ◘ Fig. 36.1) catalyses the *S*-methylation of a number of synthetic pharmacological purine analogues 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, result 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 [89, 90]). 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* [91]. Patients with no, or less efficient, methylation of thiopurines have more extensive conversion to active thionucleotides, which leads to severe, potentially fatal myelosuppression. Determination of the TPMT status is therefore now recommended prior to treatment with thiopurines [92].

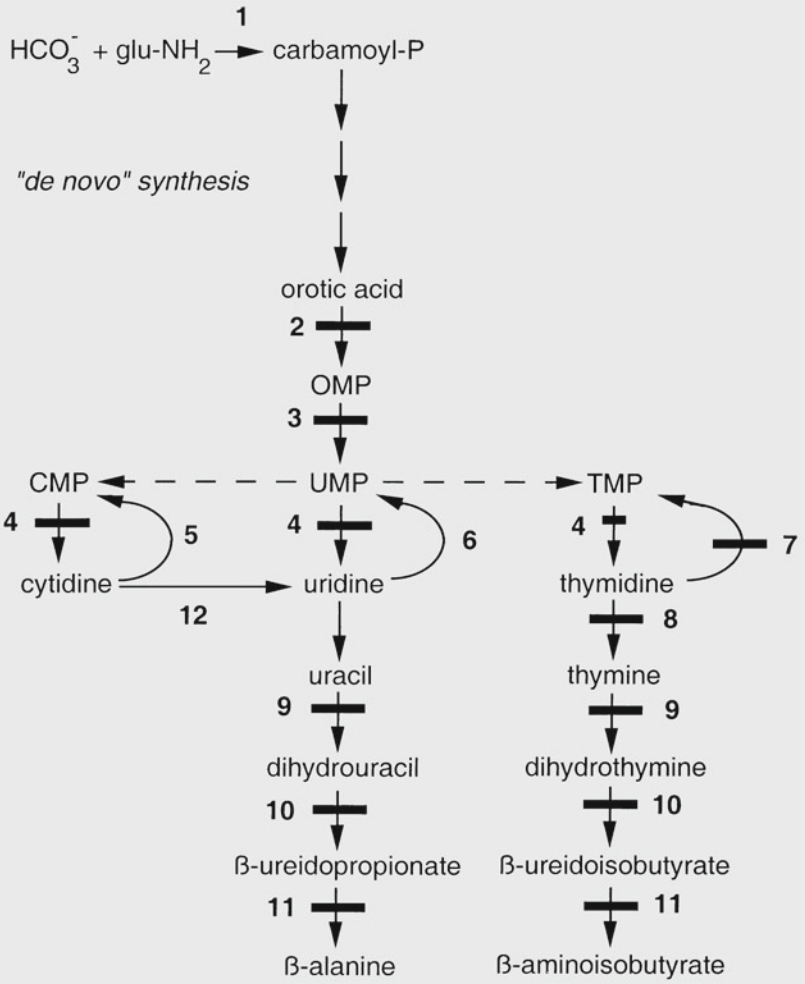
36.1.15 Inosine Triphosphatase Deficiency

Inosine triphosphate pyrophosphohydrolase (ITPase) catalyses the conversion of inosine triphosphate (ITP) to IMP and pyrophosphate (not shown in ◘ Fig. 36.1). Its activity is variably decreased, owing to genetic polymorphisms, in approximately 5% of the caucasian population. Accumulation of ITP in their RBC is the only abnormality recorded in ITPase-deficient individuals. However, since ITPase intervenes in the degradation of thio-ITP formed from pharmacological thiopurines, its deficiency might also be implicated in their accrued toxicity in some patients [90].

Pyrimidine Metabolism

Similarly to that of the purine nucleotides, the metabolism of the pyrimidine nucleotides can be divided into three pathways:

- 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 that catalyses the first step of ureogenesis (◘ 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 analogues into their active forms.



◘ **Fig. 36.3.** Pathways of pyrimidine metabolism. *CMP*, cytidine monophosphate; *glu-NH2*, glutamine; *OMP*, orotidine monophosphate; *PRPP*, phosphoribosylpyrophosphate; *TMP*, thymidine monophosphate; *UMP*, uridine monophosphate. **1**, carbamoylphosphate synthetase; **2**, orotate phosphoribosyltransferase; **3**, orotidine decarboxylase (2 and 3 form UMP synthase); **4**, pyrimidine (cytosolic) 5′-nucleotidase; **5**, cytidine kinase; **6**, uridine kinase; **7**, thymidine kinase; **8**, thymidine phosphorylase; **9**, dihydropyrimidine dehydrogenase; **10**, dihydropyrimidinase; **11**, ureidopropionase; **12**, cytidine deaminase. Enzyme deficiencies are indicated by *solid bars* across the *arrows*

36.2 Inborn Errors of Pyrimidine Metabolism

Inborn errors of pyrimidine metabolism comprise defects of:

- **Pyrimidine synthesis:** UMP synthase deficiency and Miller syndrome;
- **Pyrimidine catabolism:** deficiencies of dihydropyrimidine dehydrogenase (DPD) dihydropyrimidinase (DHP), ureidopropionase, thymidine phosphorylase (a mitochondrial disorder, see also ► Chapter 15), pyrimidine 5'-nucleotidase and cytidine deaminase and superactivity of cytosolic 5'-nucleotidase;
- **Pyrimidine salvage:** thymidine kinase 2 deficiency (a mitochondrial disease, see also ► Chapter 15).

36.2.1 UMP Synthase Deficiency (Hereditary Orotic Aciduria)

▪ Clinical Presentation

Megaloblastic anaemia, which appears a few weeks or months after birth, is usually the first manifestation [93, 94]. Peripheral blood smears often show anisocytosis, poikilocytosis and moderate hypochromia. Bone marrow examination reveals erythroid hyperplasia and numerous megaloblastic erythroid precursors. Characteristically, the anaemia does not respond to iron, folic acid or vitamin B_{12}. If unrecognised, 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. 36.3). A first reaction orotate phosphoribosyltransferase (OPRT), converts orotic acid to OMP, and a second, orotidine decarboxylase (ODC), decarboxylates OMP to UMP. In most patients, termed type I, OPRT activity was earlier claimed to be selectively defective, but later studies showed that both activities are deficient [95]. The defect provokes a massive overproduction of orotic acid, attributed to the 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. 36.3), and a deficiency of pyrimidine nucleotides [94]. The deficiency of pyrimidine nucleotides leads to impairment of cell division, which results in megaloblastic anaemia and in retardation of growth and development. In two patients who did not display megaloblastic anaemia, a selective defect of ODC activity is postulated [95].

▪ Genetics

Hereditary orotic aciduria is inherited as an autosomal recessive trait. The genetic lesion results in synthesis of an enzyme with reduced stability [96]. Three point mutations have been identified in two Japanese families [97].

▪ Diagnostic Tests

Urinary analysis reveals a massive overexcretion 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 [95]. Enzymatic diagnosis can be performed on RBC.

▪ Treatment and Prognosis

The enzyme defect can be bypassed by the administration of uridine, which is converted to UMP by uridine kinase (◘ Fig. 36.3). An initial dose of 100-150 mg/kg, divided over the day, induces prompt haematological response and acceleration of growth. The dosage should then 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.

36.2.2 Miller Syndrome

Miller syndrome is a very rare genetic condition often referred to as 'postaxial acrofacial dysostosis', which is characterised by distinctive craniofacial malformations associated with limb abnormalities. Recently, the powerful new strategy of exome sequencing has allowed identification in several patients of mutations of *DHODH*, the gene encoding dihydroorotate dehydrogenase (not shown in ◘ Fig. 36.3), which catalyses the fourth step of de novo pyrimidine synthesis [98]. Interestingly, the malformations in Miller syndrome resemble those following 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.

36.2.3 Dihydropyrimidine Dehydrogenase Deficiency

Clinical Picture

Two forms occur. The first is found in children, most of whom display epilepsy and motor and mental retardation, often accompanied by generalised hypertonia, hyperreflexia, growth delay, dysmorphic features including microcephaly, and autistic features [99]. In these patients, the deficiency of dihydropyrimidine dehydrogenase (DPD) is complete or near-complete. Nevertheless, the severity of the disorder is highly variable, and even asymptomatic cases have been identified. The second clinical picture is found in adults who receive the pyrimidine analogue 5-fluorouracil, a classic treatment for various cancers including those of the breast, ovary and colon [100, 101]. It is characterised by severe toxicity, manifested by profound neutropenia, stomatitis, diarrhoea and neurological 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 catalyses the catabolism of uracil and thymine into dihydrouracil and dihydrothymine, respectively (◘ Fig. 36.3), leads to the accumulation of the former compounds [99]. 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 of the anticancer drug 5-fluorouracil.

Genetics

The infantile form of DPD deficiency is inherited as an autosomal recessive trait. The *DPYD* gene is localised on chromosome 1, and more than 40 mutations have been identified. The most frequent are a splice site mutation (IVS14+1G>A), which results in skipping of a complete exon, and a D949V mutation [99, 101, 102]. Strikingly, patients who carry the same mutation can display widely variable clinical symptoms. In the adult form of DPD deficiency, characterised by 5'-fluorouracil toxicity, approximately 25% of patients are heterozygotes for the IVS14+1G>A mutation [101]. Recently, large genomic deletions and deep intronic mutations affecting pre-mRNA splicing have been identified in severely affected patients [103].

Diagnostic Tests

Patients excrete high amounts of uracil (56-683 mmol/mol creatinine, compared with 3-33 in control urine) and of thymine (7-439 mmol/mol creatinine, compared with 0-4 in control urine). Elevations of uracil and thymine in plasma and CSF are much less prominent [99]. 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 [20, 21] and analysis of amino acids in urine before and after acid hydrolysis [104].

The enzyme defect can be demonstrated in the patients' fibroblasts, liver and blood cells, with the exception of erythrocytes [99, 101]. In paediatric patients, DPD deficiency is complete or near-complete; in adult cancer patients experiencing acute 5-fluorouracil toxicity it is partial, with residual enzyme activities ranging from 3% to 30%.

Treatment and Prognosis

No treatment is available for paediatric 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 [100, 101].

36.2.4 Dihydropyrimidinase Deficiency

Clinical Picture

Approximately 30 patients with this disorder have been diagnosed [99, 105]. As in DPD deficiency, the clinical picture varies from severe psychomotor retardation with epilepsy, dysmorphic features or microcephaly to complete absence of any symptoms. Nearly half of the patients present with gastrointestinal problems, such as feeding difficulties, cyclic vomiting, gastro-oesophageal reflux and malabsorption.

Metabolic Derangement

Dihydropyrimidinase (DHP) catalyses the cleavage of dihydrouracil and dihydrothymine into, repectively, β-ureidopropionate and β-ureidoisobutyrate (◘ Fig. 36.3). Consequently, considerable quantities of dihydrouracil and dihydrothymine, which are normally found in small amounts, are excreted in urine [99, 105]. There is also a moderate elevation of uracil and thymine excretion. As in DPD deficiency, the reasons for the appearance and the mechanisms of the symptoms remain unexplained, and reduced concentrations of the neurotransmitters β-alanine and ß-aminoisobutyric acid may play a role. Increased sensitivity to 5-fluorouracil leading to severe toxicity has also been reported [106].

Genetics

The defect is inherited as an autosomal recessive trait. Studies of the *DPYS* gene encoding DHP, which is loca-

lised on chromosome 8, have led to the identification of a variety of mutations in symptomatic and asymptomatic individuals [105, 107]. 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 can be detected by LCMS-MS [20, 21]. Enzyme assay requires liver biopsy, since more accessible tissues do not possess DHP activity.

- **Treatment and Prognosis**

There is no therapy, and the prognosis seems unpredictable [109].

36.2.5 Ureidopropionase Deficiency

Beta ureidopropionase (also termed ß-alanine synthase) catalyses the last step of the pyrimidine degradative pathway, the conversion of ß-ureidopropionate and ß-ureidoisobutyrate to ß-alanine and ß-aminoisobutyrate, respectively (◘ Fig. 36.3). The enzyme has been found to be deficient in five patients presenting with 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 complete absence of any symptoms [109, 110, 111]. Large subdural haematomas and global supratentorial atrophy were found in a patient with an acute life-treatening status epilepticus [112]. Screening of 24,000 newborns in Japan led to the identification of four asymptomatic babies [113].

The deficiency provokes elevations of ureidopropionic acid (also called *N*-carbamoyl-β-alanine) and ureidoisobutyric acid (also called *N*-carbamoyl-β-aminoisobutyric acid), which may act as neurotoxins [114].

36.2.6 Pyrimidine 5′-Nucleotidase Deficiency

This defect is restricted to erythrocytes and leads to accumulation of pyrimidine nucleotides, resulting in basophilic stippling and chronic nonspherocytic haemolytic anaemia. Several mutations have been identified, but the mechanism by which the increased masses of pyrimidine nucleotides cause haemolysis remains unknown [115].

36.2.7 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 [116]. 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 decreases in the frequency of seizures and infections were recorded.

36.2.8 Thymidine Phosphorylase Deficiency

Patients with mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), an autosomal recessive disease associated with multiple deletions of skeletal muscle mitochondrial DNA (see also ▶ Chapter 15), have been shown to be deficient in thymidine phosphorylase owing to a variety of mutations [117]. The enzyme deficiency results in marked accumulation of thymidine, which most probably provokes an imbalance of the mitochondrial nucleotides and hence compromises the replication of mitochondrial DNA. Allogeneic haematopoietic stem cell transplantation has been performed in a number of patients, with varying success [118].

36.2.9 Cytidine Deaminase Deficiency

Cytidine deaminase catalyses the conversion of cytidine into uridine along the pyrimidine catabolic pathway (◘ Fig. 36.3). It plays a key role in the inactivation of gemcitabine, a cytidine analogue with activity against solid tumours. 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 [119] 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 the autosomal recessive form of the hyper-IgM syndrome [120].

36.2.10 Thymidine Kinase 2 Deficiency

Initially described in four unrelated patients with very severe, isolated myopathy, motor regression and early death [121], 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 [122]. The deficiency results in depletion of muscular mitochondrial DNA (see also ►Chapter 15) and is caused by mutations of the gene encoding thymidine kinase 2, the mitochondrial form of this thymidine salvage enzyme [121, 123]. As in the deficiencies of deoxyguanosine kinase and thymidine phosphorylase, the defect probably 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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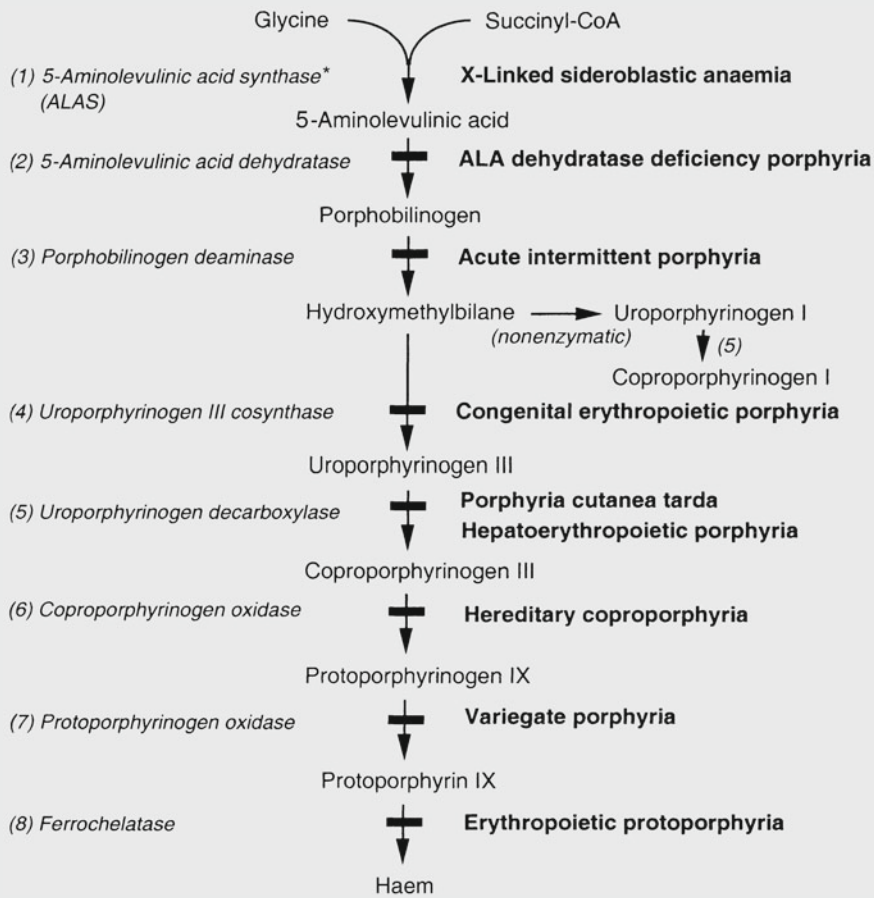
The Haem Biosynthetic Pathway

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. 37.1) consists of eight enzymes and their substrates and products. The first and last three enzymes are located in the mitochondria and the other four, in the cytosol.

The pathway is regulated differently in bone marrow and liver, which are the tissues that make the largest amounts of haem. The first enzyme of the pathway, 5-aminolevulinic acid synthase (ALAS), is the only enzyme in this pathway for which erythroid and nonerythroid (housekeeping) forms of the enzyme are encoded by separate genes. The nonerythroid 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 [1, 2]. By contrast, the erythroid-specific enzyme (ALAS2), which is encoded by a separate gene located on the X chromosome, is induced by haem and erythropoietin. The *ALAS2* gene and those for at least three other enzymes of the pathway contain DNA sequences that provide for erythroid-specific regulation of haem synthesis. Therefore, factors that influence liver cytochrome P450 enzyme synthesis, such as drugs, diet and certain hormones, have little effect on haemoglobin synthesis in the bone marrow.



◘ **Fig. 37.1.** Pathway of haem biosynthesis. Intermediates and enzymes of the haem biosynthetic pathway are listed. *ALA*, 5-Aminolevulinic acid; *CoA*, coenzyme A. The porphyrias caused by the various enzyme deficiencies (indicated by *solid bars* across the *arrows*) are given in *bold*. *Mutation with gain of function causes the X-linked variant of erythropoietic protoporphyria

X-Linked sideroblastic anaemia is due to a deficiency of the erythroid form of the first enzyme in the haem biosynthetic pathway, 5-aminolevulinic acid synthase. 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 other 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, *porphyria cutanea tarda, acute intermittent porphyria* and *erythropoietic protoporphyria*, differ considerably from each other. 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, although the skin manifestations and methods of diagnosis and treatment are very different. Porphyrias are more often manifest in adults than are most metabolic diseases. All porphyrias are inherited, with the exception of porphyria cutanea tarda, which is due to an acquired enzyme deficiency in liver, although an inherited deficiency is a predisposing factor in some cases.

37.1 X-Linked Sideroblastic Anaemia

■ Clinical Presentation

Sideroblastic anaemia can be either acquired or inherited. 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 [3].

■ Metabolic Derangement

These features reflect a deficiency of protoporphyrin and 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 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 is due to loss-of-function mutations of the *ALAS2* gene. Cases of sideroblastic anaemia due to mutations of SLC25A38, a mitochondrial transporter, have also been described [4]. Phenotype and genotype of the X-linked disorder are heterogeneous [5-7]. Point mutations may 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.

■ 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. Screening for *HFE* mutations may identify patients at greater than expected 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 [3].

37.2 The Porphyrias

These metabolic disorders are due to 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 (also known as δ-aminolevulinic acid) and porphobilinogen

The patterns of excess intermediates in these disorders are complex, but usually characteristic for each type of porphyria. 5-aminolevulinic acid and porphobilinogen are water soluble, colourless and nonfluorescent and are excreted almost entirely in urine, as are porphyrins with a large number of carboxyl side chains (e.g. uroporphyrin, an octacarboxyl porphyrin). Protoporphyrin (a dicarboxyl porphyrin) is not soluble in water and is excreted entirely in bile and faeces. Coproporphyrin (a tetracarboxyl 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 auto-oxidation if they leave the intracellular environment and are then excreted primarily as the corresponding

porphyrins, which are reddish and fluorescent when exposed to ultraviolet light [8-10].

37.2.1 Classification and Diagnosis

The porphyrias are classified according to the tissue where intermediates initially accumulate (hepatic and erythropoietic porphyrias), or to the clinical presentation (acute neurovisceral or cutaneous porphyrias) (◘ Table 37.1).

Four porphyrias are associated with striking increases in 5-aminolevulinic acid, and three 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. The two most common cutaneous porphyrias are porphyria cutanea tarda and erythropoietic protoporphyria. Variegate porphyria and hereditary coproporphyria can cause either neurological or cutaneous symptoms.

A diagnosis of porphyria should be considered in patients with unexplained neurovisceral symptoms, such as abdominal pain, or cutaneous photosensitivity. It is very important to confirm or exclude this diagnosis promptly, because treatment is more successful if started soon after the onset of symptoms. Inherited 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. In contrast to the nonspecific nature of symptoms, laboratory tests, if properly chosen and interpreted, can be both sensitive and specific [11]. 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 37.2). 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 the 5-aminolevulinic acid and total porphyrins be measured later on the same sample [12]. Indeed, the finding of normal levels of these compounds excludes all acute porphyrias as potential causes of current symptoms.

◘ **Table 37.1.** Diseases associated with alterations in enzymes in the haem biosynthetic pathway and 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

		Porphyria classification			
Disease	**Enzyme**	**Hepatic**	**Erythro-poietic**	**Acute**	**Cutaneous**
X-Linked sideroblastic anaemia X-Linked protoporphyria	5-Aminolevulinic acid synthase, erythroid form	NA	NA X	NA	NA X
5-Aminolevulinic acid dehydratase porphyria	5-Aminolevulinic acid dehydratase	? X		X	
Acute intermittent porphyria	Porphobilinogen deaminase[1]	X		X	
Congenital erythropoietic porphyria	Uroporphyrinogen III cosynthase		X		X
Porphyria cutanea tarda	Uroporphyrinogen decarboxylase[2]	X			X
Hepato-erythropoietic porphyria	Uroporphyrinogen decarboxylase	X	X		X
Hereditary coproporphyria	Coproporphyrinogen oxidase	X		X	X
Variegate porphyria	Protoporphyrinogen oxidase	X		X	X
Erythropoietic protoporphyria	Ferrochelatase		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

Table 37.2. First-line laboratory tests for screening for porphyrias and second-line tests for further evaluation when initial testing is positive

Symptoms suggesting porphyria		
Testing	**Acute neurovisceral symptoms**	**Cutaneous photosensitivity**
First-line	Urinary 5-aminolevulinic acid, porphobilinogen[1] and total porphyrins[2] (quantitative; random or 24-h urine)	*Blistering skin lesions*: Total plasma porphyrins.[3] *Nonblistering*: Erythrocyte porphyrins[4]
Second-line	Urinary 5-aminolevulinic acid, porphobilinogen and total porphyrins[2] Total faecal porphyrins[2] Erythrocyte porphobilinogen deaminase Total plasma porphyrins[3] Mutation analysis	Erythrocyte porphyrins Urinary 5-aminolevulinic acid, porphobilinogen and total porphyrins[2] Total faecal porphyrins[2] Mutation analysis

[1]A kit is available for rapid, semiquantitative screening for elevated urine porphobilinogen. [2]Fractionation of urinary and faecal porphyrins is usually not helpful unless the total is increased. [3]The preferred method is direct fluorescence spectrophotometry. [4]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

Total plasma porphyrins are increased in virtually all patients with blistering skin lesions due to porphyrias, and should be measured when a cutaneous porphyria is suspected, preferably by a direct fluorometric method [11, 12]. Measurement of erythrocyte protoporphyrin is necessary for the diagnosis of erythropoietic protoporphyria, which causes nonblistering photosensitivity.

Further laboratory evaluation is necessary if the initial tests are positive, in order to distinguish between the different types of porphyria.

37.3 5-Aminolevulinic Acid Dehydratase Porphyria

Clinical Presentation

Only six cases of this porphyria have been documented by molecular methods. 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-aminolevulinic acid dehydratase, the second enzyme in the haem biosynthetic pathway (Fig. 37.1) [13, 14] (Table 37.1). This porphyria is commonly classified as hepatic, along with the other acute porphyrias, but the marrow may be an important site of 5-aminolevulinic acid overproduction in this disease.

Lead poisoning can be distinguished by showing reversal of the inhibition of 5-aminolevulinic acid dehydratase in erythrocytes by the in vitro addition of dithiothreitol. Hereditary tyrosinaemia type 1, resulting from a deficiency of fumarylacetoacetase, leads to accumulation of succinylacetone (2,3-dioxoheptanoic acid), a potent inhibitor of 5-aminolevulinic acid dehydratase (► Chapter 18). Other heavy metals and styrene can also inhibit this enzyme.

Genetics

This is an autosomal recessive disorder; enzyme activity is approximately half normal 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 increases in urinary 5-aminolevulinic acid and coproporphyrin and erythrocyte zinc protoporphyrin, normal or only slightly increased urinary porphobilinogen, and a marked decrease in erythrocyte 5-aminolevulinic acid dehydratase activity. Excess coproporphyrin III probably results from metabolism of 5-aminolevulinic acid via the haem biosynthetic pathway in a tissue other than the site of its initial accumulation [15]. Erythrocyte zinc protoporphyrin content is also increased, as in other homozygous cases of porphyria. The diagnosis of this porphyria should be confirmed by DNA studies [13].

▪ Treatment and Prognosis

There is little experience in treating this porphyria. 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.

37.4 Acute Intermittent Porphyria

▪ Clinical Presentation

This autosomal dominant condition is the most common of the acute porphyrias, with a prevalence estimated at approximately 5 per 100,000 in northern European populations. 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. 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 suggest sympathetic overactivity. Other common manifestations may include nausea, vomiting, constipation, pain in the limbs, head, neck or chest, muscle weakness and sensory loss [16]. 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 characteristically 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 due to other causes unrelated to 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 [17].

Persistent hypertension and impaired renal function may occur 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 [9, 19, 20].

▪ Metabolic Derangement

Acute intermittent porphyria is due to reduced activity of porphobilinogen deaminase (also known as hydroxymethylbilane synthase and formerly as uroporphyrinogen I synthase), (◘ Fig. 37.1, ◘ Table 37.1). 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. Therefore, acute intermittent porphyria is classified as one of the hepatic porphyrias.

When drugs, hormones, or nutritional factors increase the demand for hepatic haem by having the capacity to induce the synthesis of cytochrome P450 enzymes and ALAS1 in the liver [2], the deficient enzyme can become limiting for haem synthesis. Induction of hepatic ALAS1 is then accentuated and 5-aminolevulinic acid and porphobilinogen accumulate. Excess porphyrins originate nonenzymatically from porphobilinogen, and perhaps enzymatically from 5-aminolevulinic acid transported to tissues other than the liver [2].

▪ Genetics

Inheritance is autosomal dominant; more than 300 different mutations of the porphobilinogen deaminase gene have been identified in unrelated families [20]. Two forms of this enzyme, an erythroid-specific and a nonerythroid (housekeeping) form are derived from the same gene. A deficiency of the housekeeping form in the liver is essential for causing acute intermittent porphyria. Mutations located in or near the first of the 15 exons in this gene can impair the synthesis of the housekeeping form but not of the erythroid-specific form of porphobilinogen deaminase. Homozygous cases of acute intermittent porphyria are extremely rare, but should be suspected in early childhood cases [20, 23].

▪ Diagnostic Tests

The finding of a substantial increase in urinary porphobilinogen is a sensitive and specific indication that a patient has either acute intermittent porphyria, hereditary coproporphyria or variegate porphyria (◘ Table 37.2). A kit is available for the rapid detection of porphobilinogen at concentrations greater than 6 mg/l, and provides a colour chart for semiquantitative estimation of higher levels [22].

This enables rapid 'in-house' testing for these disorders [11]. Porphobilinogen usually remains increased between attacks of acute intermittent porphyria unless there have been no symptoms for a prolonged period. Faecal total porphyrins are generally normal or minimally increased in acute intermittent porphyria, 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 acute intermittent porphyria. Urinary coproporphyrin is generally more increased in hereditary coproporphyria and variegate porphyria than in acute intermittent porphyria. Urinary uroporphyrin can be increased in all these disorders, especially when porphobilinogen is increased.

Decreased erythrocyte porphobilinogen deaminase helps to confirm a diagnosis of acute intermittent porphyria. Limitations of this measurement include falsely low activity with suboptimal processing or storage of the sample and the wide normal range (up to 3-fold) that overlaps the range of patients with acute intermittent porphyria.

Measuring erythrocyte porphobilinogen deaminase can be useful for detecting asymptomatic carriers, but DNA studies are preferred [9, 23].

▪ Treatment and Prognosis

Intravenous haemin (haem arginate or haematin 3-4 mg per kg body weight infused i.v. once daily for 4 days) is considered specific therapy for acute attacks of porphyria, because it represses hepatic ALAS1 and markedly reduces levels of aminolevulinic acid and porphobilinogen [9]. 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 [24]. 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 [25, 27].

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. Diazepam in low doses is safe if a minor tranquilliser is required. Bladder distension may require catheterisation.

Intravenous glucose can be tried instead of haemin for mild attacks. At least 300 g daily is recommended, and >500 g daily may be more effective. If tolerated, oral carbohydrate loading is also an option.

With resolution of an attack abdominal pain may disappear within hours, and paresis begins to improve within days. Muscle weakness may resolve gradually and completely even after a prolonged attack with severe motor neuropathy, but there may be some residual weakness.

Treatment of seizures is problematic, because almost all antiseizure drugs can exacerbate acute porphyrias. Bromides, gabapentin, levetiracetam and probably vigabatrin can be given safely [26]. β-Adrenergic blocking agents may control tachycardia and hypertension in acute attacks of porphyria [27]. Progressive renal disease may develop and may be accompanied by increased plasma porphyrins and blistering photosensitivity [18]. Renal failure may be treated by haemodialysis or renal transplantation [29, 30].

Allogeneic liver transplantation for patients with frequent attacks refractory to haemin is effective if accomplished before there is advanced motor paralysis [29, 30]. Some patients with renal failure have undergone combined liver and renal transplantation [30].

Identification and correction of precipitating factors such as harmful drugs, dietary indiscretions, cyclic endogenous or exogenous hormones (particularly progesterone and progestins) and intercurrent infections can hasten recovery from an attack and prevent future attacks. Very frequent cyclic 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 [31].

With prompt treatment and precautions to prevent further attacks, the outlook for patients with acute porphyrias is usually excellent [19]. 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.

37.5 Congenital Erythropoietic Porphyria (Gunther Disease)

▪ Clinical Presentation

This is usually a severe disease with manifestations noted soon after birth, or even in utero. Rarely, symptoms 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 may fluoresce when exposed to long-wave ultraviolet light. Porphyrins are also deposited in bone. Haemolysis is almost invariably present, resulting from 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.

Congenital erythropoietic porphyria can present in utero as nonimmune hydrops [32]. 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 cosynthase (or synthase) (◘ Fig. 37.1, ◘ Table 37.1). 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. Therefore, uroporphyrin, coproporphyrin and other porphyrins accumulate in bone marrow, plasma, urine and faeces. This accumulation in erythroid cells results in intramedullary and intravascular haemolysis, which leads to increased erythropoiesis. As a result, haem synthesis is actually increased in spite of the inherited enzyme deficiency, in order to compensate for porphyrin-induced haemolysis. 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 [33].

▪ Genetics

Congenital erythropoietic porphyria is an autosomal recessive disorder. Patients have either homozygous or compound heterozygous mutations of the gene that encodes uroporphyrinogen III cosynthase. Like other porphyrias, this disease is genetically heterogeneous, and at least 39 different cosynthase mutations and a GATA-1 mutation have been identified [34, 35]. Parents and other heterozygotes display intermediate deficiencies of the enzyme.

▪ Diagnostic Tests

Erythrocyte and plasma porphyrins are markedly increased and usually consist mostly of uroporphyrin I. Coproporphyrin 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. The diagnosis should be confirmed by a markedly deficient uroporphyrinogen III cosynthase activity and by mutation analysis. The disease can be diagnosed in utero by porphyrin measurements and DNA studies.

▪ Treatment and Prognosis

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. Improvement in haemolysis has been reported 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 [36, 37, 38]. Marrow or stem cell transplantation is the most effective current therapy [39, 40], and gene therapy may eventually be possible [41].

37.6 Porphyria Cutanea Tarda

▪ Clinical Presentation

This is the most common and readily treated form of porphyria and is manifested primarily by chronic, blistering skin lesions, especially on the backs of the hands, forearms, face and (in women) the dorsa of the feet. Neurological effects are not observed. Sun-exposed skin is also 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 protoporphyria (► see later discussion).

A number of acquired and inherited susceptibility factors contribute to the development of porphyria cutanea tarda. A normal or increased amount of hepatic iron is a requirement for the disease. Others include moderate or heavy alcohol intake, hepatitis C infection, oestrogen use and smoking. Infection with HIV is a less common association. There are geographic differences in the association with hepatitis C; in some locations up to 80% of patients are infected with this virus. Porphyria cutanea tarda developed after ingestion of seed wheat treated with

hexachlorobenzene as a fungicide and after occupational exposure to related chemicals such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, dioxin). However, such toxic exposures are seldom evident in isolated cases of porphyria cutanea tarda [42, 43].

▪ Metabolic Derangement

This porphyria is caused by a profound deficiency of hepatic uroporphyrinogen decarboxylase (UROD) (◘ Fig. 37.1, ◘ Table 37.1). 'Sporadic' (type 1) and 'familial' (types 2 and 3) forms of the disease have been described, which do not differ substantially in terms of clinical features or treatment. In all cases, a specific inhibitor of hepatic UROD, which has been recently characterised as a uroporphomethene [44], is generated from an intermediate of the haem biosynthetic pathway (probably uroporphyrinogen) by an iron-dependent oxidative mechanism. Susceptibility factors, including alcohol, hepatitis C, oestrogens, smoking, certain cytochrome P450 enzymes, HIV and low levels of ascorbic acid and carotenoids, may contribute to this oxidative process within hepatocytes. The prevalence of HFE mutations is increased in this disease [43]. HFE mutations, alcohol and hepatitis C dysregulate hepcidin production by the liver, thereby increasing iron absorption. Individuals with type 2 disease have only a half-normal amount of the enzyme protein from birth onward and are therefore more susceptible to sufficient inhibition of the hepatic enzyme to cause the disease [42, 43].

Patterns of excess porphyrins in this disease are complex and characteristic. Uroporphyrinogen and its derivatives the hepta-, hexa- and pentacarboxyl porphyrinogens accumulate and are oxidised to the corresponding porphyrins. To complicate the porphyrin pattern further, pentacarboxyl porphyrinogen can be metabolised by coproporphyrinogen oxidase (the next enzyme in the pathway) to a tetracarboxyl porphyrinogen termed isocoproporphyrinogen. All these compounds appear in plasma and are excreted in urine, bile and faeces as the oxidised porphyrins. Successful treatment may require some time before the massive porphyrin accumulations in liver are cleared.

▪ Genetics

Porphyria cutanea tarda results from an acquired deficiency of hepatic UROD. Genetic factors contribute in some patients, including an inherited partial deficiency of this enzyme. In type 1 porphyria cutanea tarda there are no UROD mutations and the amount of hepatic UROD protein is normal.

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. Type 2 becomes clinically manifest when hepatic UROD becomes profoundly inhibited, as in type 1. Type 2 does not differ clinically from type 1 except in that there is occasionally a family history of porphyria cutanea tarda and earlier onset of manifest disease. Susceptibility factors in both are similar, and both are responsive to treatment More than 100 mutations have been identified in type 2 disease. Cases classified as type 3 disease, which are rare, have normal erythrocyte UROD activity, but one or more relatives also have the disease. A genetic defect has not been identified in type 3, and it is possible that these cases are not fundamentally different from type 1 [44].

▪ Diagnostic Tests

Blistering skin lesions are not specific, and are found in all cutaneous porphyrias, except erythropoietic protoporphyria. Skin histopathology is also not specific. Therefore, a skin biopsy does not establish a diagnosis of porphyria cutanea tarda or exclude pseudoporphyria. It is important to differentiate these conditions by laboratory testing before therapy.

Plasma and urine porphyrins are increased in all patients with blistering skin lesions due to porphyria. The fluorescence spectrum of plasma porphyrins can rapidly distinguish variegate porphyria from porphyria cutanea tarda (◘ Table 37.2). The diagnosis of porphyria cutanea tarda is best confirmed by increased total urinary or plasma porphyrins with a predominance of highly carboxylated porphyrins, especially uroporphyrin and heptacarboxyl porphyrin. Cases of so-called pseudoporphyria have skin lesions resembling porphyria cutanea tarda 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. 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 1- to 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 fall more slowly than ferritin, and may not yet be normal when the target ferritin level is reached.

With treatment hepatic UROD activity gradually increases to normal. After remission, ferritin may increase without recurrence, in most cases. Postmenopausal women who have been treated for porphyria cutanea tarda can usually resume oestrogen replacement without recurrence. 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 50 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) for several months 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. 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 known [46], but they are not effective in other porphyrias [27].

37.7 Hepatoerythropoietic Porphyria

▪ Clinical Presentation

This rare disease is clinically similar to congenital erythropoietic porphyria and usually presents with red urine and blistering skin lesions shortly after birth. Mild cases may present later in life and more closely resemble porphyria cutanea tarda. Concurrent conditions, such as viral hepatitis, may accentuate porphyrin accumulation.

▪ Metabolic Derangement

Hepatoerythropoietic porphyria is the homozygous form of familial (type 2) porphyria cutanea tarda and is due to a substantial deficiency of uroporphyrinogen decarboxylase (UROD) [47]. Intermediate deficiencies of the enzyme are found in the parents (◘ Fig. 37.1, ◘ Table 37.1). The disease has features of both hepatic and erythropoietic porphyrias. Although it is usually a more severe disease than porphyria cutanea tarda, there are reports of mild and atypical forms of the disease [48].

▪ Diagnostic Tests

The excess porphyrins found in urine, plasma and faeces in this condition are similar to those in porphyria cutanea tarda. In addition, erythrocyte zinc protoporphyrin is 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. Erythrocyte porphyrins in congenital erythropoietic porphyria 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 a homozygous or compound heterozygous state for mutations of the gene encoding UROD. 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 [47].

▪ Treatment and Prognosis

Therapeutic options are essentially the same as in congenital erythropoietic porphyria.

37.8 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 acute intermittent porphyria. However, unlike the latter disease, they are also cutaneous porphyrias, because they may cause blistering skin lesions that are indistinguishable from those of porphyria cutanea tarda. Factors that exacerbate acute intermittent porphyria 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 where most cases are observed in descendants of a couple who emigrated from Holland and arrived in Cape Town in 1688 [49].

▪ Metabolic Derangement

Hereditary coproporphyria and variegate porphyria result from deficiencies of coproporphyrinogen oxidase and of protoporphyrinogen oxidase, respectively, (◘ Fig. 37.1, ◘ Table 37.1). 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 bile) 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 porphobilinogen deaminase, which along with induction of hepatic ALAS1, may account for the increase in porphyrin precursors during acute attacks of these conditions [50].

▪ Genetics

Both of these porphyrias are autosomal dominant conditions in which affected individuals and latent carriers have approximately 50% activity of the affected enzyme. Homozygous cases are rare. 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 [49].

▪ Diagnostic Tests

Urinary 5-aminolevulinic acid and porphobilinogen are increased during acute attacks of these porphyrias, although the increases may be smaller and more transient than in acute intermittent porphyria. Urinary coproporphyrin increases may be more prominent and prolonged, 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 the other porphyrias [50-53].

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. Mutation analysis is available [53].

▪ Treatment and Prognosis

Attacks of neurological symptoms are treated as in acute intermittent porphyria (see ► above). Cutaneous symptoms are more difficult to treat, and therapies that are effective for porphyria cutanea tarda (phlebotomy and low-dose hydroxychloroquine) are not effective in these conditions. Protection from sunlight is important.

37.9 Erythropoietic Protoporphyria

▪ Clinical Presentation

Erythropoietic protoporphyria is the third most common porphyria, and the most common in children. Cutaneous symptoms, which usually begin in early childhood, are generally much more prominent than objective changes by examination. Symptoms such as pain and itching, sometimes with erythema and swelling can occur within minutes of sun exposure, and the diffuse oedema of sun-exposed areas may resemble angioneurotic oedema. Chronic skin changes may include lichenification, leathery pseudovesicles, labial grooving and nail changes, but may be 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 may reflect down-regulation of iron absorption [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 [56, 57]. 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 [58].

▪ Metabolic Derangement

This disease is due either to an inherited deficiency of ferrochelatase, the eighth and last enzyme in the haem biosynthetic pathway, or much less commonly to a gain-of-function mutation of ALAS2, the erythroid form of the first enzyme in the pathway (◘ Fig. 37.1, ◘ Table 37.1). Ferrochelase deficiency, if substantial (10-30% of normal), leads to increases in protoporphyrin in bone marrow, circulating erythrocytes, plasma, bile and faeces in

this disease. The deficient enzyme is rate limiting for protoporphyrin metabolism primarily in bone marrow reticulocytes, which are the primary source of the excess protoporphyrin. Circulating erythrocytes, and perhaps the liver, contribute smaller amounts. Excess protoporphyrin is transported in plasma and excreted in bile and faeces. Zinc protoporphyrin is also a product of ferrochelatase activity, so that in this disease the excess protoporphyrin found in erythrocytes is mostly metal free. Young circulating erythrocytes appear fluorescent when a blood smear from a patient with this condition is examined by fluorescence microscopy.

In the X-linked variant form of this disease without ferrochelatase mutations, the proportion of zinc protoporphyrin in erythrocytes is greater (15~50% of the total) than in cases with ferrochelatase deficiency (0~15%). This X-linked form is caused by gain-of-function mutations of the *ALAS2* gene, which is located on the X chromosome [59-61]. The phenotype of X-linked protoporphyria is identical to erythropoietic protoporphyria, except for the greater proportion of zinc protoporphyrin in erythrocytes, although it has been suggested that liver disease may be more common in the X-linked form [62].

Protoporphyrin is excreted in bile and may undergo enterohepatic circulation. Large amounts of protoporphyrin can cause cholestasis and hepatocellular damage, which may explain the development of liver failure in some patients with protoporphyria. In these patients, the excess protoporphyrin deposited in the liver appears to be derived primarily from the bone marrow.

Genetics

At least 125 mutations in the ferrochelatase gene have been identified in protoporphyria [60, 61], and most mutant alleles express little or no ferrochelatase (disabling or null mutations. However, most patients with severe ferrochelatase mutations and clinically manifest disease have also inherited a weak, or hypomorphic, ferrochelatase allele [63, 64]. This allele is found in ~10% of normal Caucasians, and has no consequence unless *trans* to a mutant ferrochelatase allele that results in little or no enzyme activity [64]. Therefore, ferrochelatase activity is only 10-30% of normal in patients with manifest disease, rather than the expected ~50% for autosomal dominant inheritance. This pattern of inheritance, which is found in the great majority of cases, is best regarded as autosomal recessive at the molecular level. A few families have been described with two disabling mutations, where at least one of the two mutant alleles expresses some ferrochelatase activity [55, 62]. For unknown reasons, seasonal palmar keratoderma is common in some of these families [63]. Late-onset protoporphyria may develop in the presence of myeloproliferative disorders, with clonal expansion of erythropoietic cells with a ferrochelatase gene mutation or deletion [64].

In families with X-linked protoporphyria, the disease is likely to be more common and severe in male and more variable in female family members, presumably reflecting the degree of X chromosome inactivation.

Diagnostic Tests

The most sensitive screening test for this disorder is a determination of total erythrocyte protoporphyrin, although this test lacks specificity. The result is usually expressed as protoporphyrin because this is the predominant erythrocyte porphyrin in most circumstances. An increased erythrocyte total protoporphyrin result must be followed by fractionation to discern whether the protoporphyrin is free or complexed with zinc. Fractionation of the individual porphyrins by HPLC is required when congenital erythropoietic porphyria is suspected.

The plasma porphyrin concentration is increased in almost all cases, but less so than in other cutaneous porphyrias. 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. It is especially important to protect plasma samples from light if protoporphyria is suspected. [51, 53].

Total faecal porphyrins may be normal or increased in protoporphyria, with a predominance of protoporphyrin. Urinary porphyrins and porphyrin precursors are normal, unless the patient has liver impairment.

Treatment and Prognosis

Photosensitivity is managed by avoidance of sunlight. Oral β-carotene and cysteine improve tolerance to sunlight in some patients, perhaps by quenching singlet oxygen or free radicals. Afamelanotide, a synthetic agonistic analogue of alpha melanocyte-stimulating hormone, is under development for treatment of erythropoietic protoporphyria. This drug increases melanin formation and darkens the skin, and in early studies has increased sunlight tolerance [65, 66]. Narrow-band ultraviolet light therapy is another option for increasing skin pigmentation. Cholestyramine may reduce protoporphyrin levels by interrupting its enterohepatic circulation, but has not been extensively studied [67]. Iron deficiency, caloric restriction, and drugs or hormone preparations that impair hepatic excretory function should be avoided. 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 [55].

Treatment of liver complications is difficult. Transfusions and intravenous haemin may suppress erythroid and

hepatic protoporphyrin production. Plasma exchange, cholestyramine, ursodeoxycholic acid and vitamin E are also administered. Liver transplantation is sometimes required [68]. Sequential bone marrow transplantation can prevent recurrence of hepatopathy [69, 70].

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IX Disorders of Metal Transport

Disorders in the Transport of Copper, Iron, Magnesium, Manganese, Selenium and Zinc

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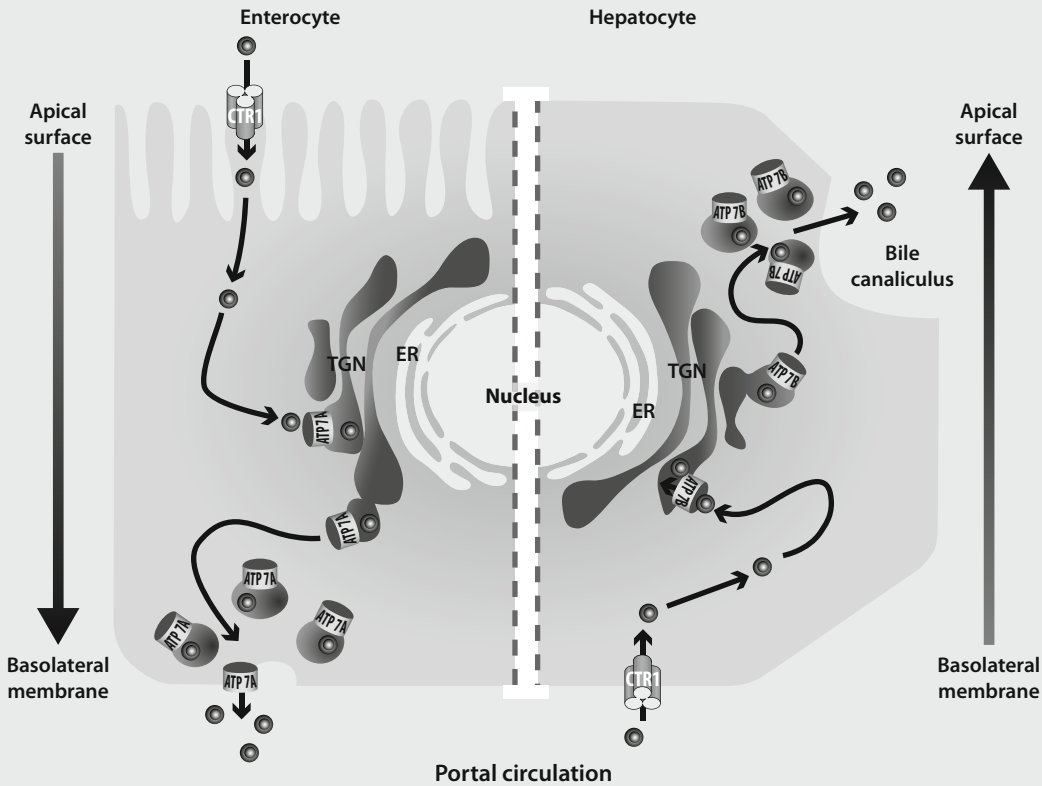
J.-M. Saudubray et. al (Eds.), *Inborn Metabolic Diseases,* DOI 10.1007/978-3-642-15720-2_38,

Copper Metabolism

Each day, about 2 mg of copper is absorbed from the intestine. Copper enters the enterocytes (◘ Fig. 38.1, left part) at their apical surface through CTR1, its main transporter, and is transferred to ATP7A, an ATPase which is synthesised in the endoplasmic reticulum (ER) and stored in the trans-Golgi network (TGN). With increasing intracellular copper, ATP7A relocalises to the basolateral membrane and releases copper in the portal circulation.

In the hepatocytes (◘ Fig. 38.1 [1], right part) copper, taken up from the portal circulation by the transporter CTR1, localises to the closely related ATP7B, and is excreted in the bile canaliculi, at their apical surface [1].

Excretion of copper by the liver into the bile is the only mechanism of elimination, and normally the amount excreted is equivalent to that taken up from the intestine. As copper, which is essential for cellular metabolism, is 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 plasma, over 90% of copper is bound to ceruloplasmin.



◘ **Fig. 38.1.** Uptake and release of copper in intestine and liver. *ER*, endoplasmic reticulum, *TGN*, *trans*-Golgi network. (Modified from [1])

38

Copper balance is disturbed in two inborn errors: Wilson disease and Menkes disease. *Wilson disease*, or hepatolenticular degeneration, is caused by mutations in the ATP7B gene and is characterised by a gradual accumulation of copper in the liver and, secondarily, in other organs, such as brain, kidney and cornea. Clinical symptoms result from 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 characterised 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.

38.1 Copper

38.1.1 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, 3]. 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, are generally much lower than in autoimmune or viral hepatitis [4]. 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 2nd or 3rd 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 deterioration in the quality of handwriting. In some patients psychiatric symptoms predominate, ranging from behavioural disturbances, often characterised 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 gallstones, 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 presymptomatic 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 oxidise the mitochondrial membranes, resulting in their swelling and loss of oxidative phosphorylation capacity. The characteristic Kayser-Fleischer ring is a deposit of copper and sulfur. The renal dysfunction is a consequence of copper accumulation in the renal tubules. The increased urinary copper excretion characteristic of 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 localised 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 [5].

▪ Genetics

Wilson disease in an autosomal recessive condition caused by mutations in the *ATP7B* gene, localised on chromosome 13q14 [6]. 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.

More than 300 mutations in the *ATP7B* gene 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 [7], the H1069Q mutation amongst European patients [8], and still other mutations being prevalent elsewhere. Most patients are compound heterozygotes. Mutations that completely destroy the function of the protein are

generally found in patients who present early, while residual function is associated with late presentation. For example, patients homozygous for the nonfunctional R778L mutation tend to present earlier with hepatic manifestations [7], 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 [8].

Diagnostic Tests

Wilson disease is characterised by low serum ceruloplasmin and serum copper, elevated urinary copper, and increased liver copper (Table 38.1). These laboratory results should only be interpreted in combination, because each individual parameter can be abnormal in situations other than Wilson disease [9]. 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 aceruloplasminaemia. Conversely, serum ceruloplasmin is normal in a small proportion of patients with Wilson disease.

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 milligram 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. The diagnostic value of this parameter might be improved by administering a loading dose of penicillamine.

When Wilson disease is diagnosed in a family, siblings should be investigated. Analysis of mutations, or using closely linked markers, 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.

Table 38.1. Laboratory results in Wilson disease patients 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

Treatment and Prognosis

The prognosis is excellent for patients who start treatment before severe tissue damage has occurred, i.e. while presymptomatic or following diagnosis at an early stage [10]. 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. Tetrathiomolybdate is a relative new agent and experience is limited so far.

Since it was the first agent to be introduced, penicillamine is the one for which the largest experience is available. Penicillamine chelates copper by forming a stable complex that is subsequently excreted in urine. The initial dose for adults is 1-2 g/day, divided into four doses, together with 25 mg/day of pyridoxine. With this therapy the majority of patients with liver disease will recover, although liver transplantation cannot be avoided in all cases [10]. Of patients with neurological disease 80% will recover, but the majority will have some residual disabilities. In the remainder no improvement is seen, and there may even be deterioration of symptoms. In this group mortality is not uncommon [10]. A significant proportion of patients with neurological disease will have initial worsening of symptoms after starting penicillamine therapy. For these patients the chances of recovery are lower. 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 [10]. 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 that of penicillamine, albeit with fewer side effects [11]. The initial dose is 1200-1800 mg/day in adults, divided into two to three doses, with subsequent maintenance therapy of 900-1200 mg/day [3].

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, the copper balance will become negative through faecal excretion, as villus cells are lost into the intestinal lumen. Compared with penicillamine, zinc does not have any serious side effects, although some patients experience gastric complaints on zinc sulfate. 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 symp-

tomatic disease (particularly with neurological symptoms) a small nonrandomised, nonblinded trial showed similar outcomes for zinc and penicillamine [12]. 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 [10]. In patients with hepatic disease zinc seems less appropriate [13]. Obviously, more trials are needed before final conclusions can be drawn. The initial dose of zinc sulfate for adults is 600 mg/day, divided into three doses; this dose can be doubled if insufficient effect is obtained. Urinary copper excretion should be monitored: it should fall rapidly initially, and more slowly thereafter. A reasonable goal is to achieve excretion of below 2 μmol/day. Copper depletion should be avoided: in the maintenance phase, 300 mg/day of zinc sulfate or even less can be sufficient.

Tetrathiomolybdate, a copper-chelating agent with greater affinity for copper than penicillamine, has been used mainly for initial decoppering of patients with neurological symptoms [14]. The initial deterioration often seen in patients treated with penicillamine appears to occur less frequently.

In patients presenting with severe liver disease, sufficient experience is only available for penicillamine. In this group the revised King's score will accurately predict who will recover and who will need a liver transplant [15]. 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), tetrathiomolybdate or the addition of high-dose vitamin E to the copper-chelating therapy, as this might protect the liver mitochondria against oxidative damage. Recently it was also suggested that amitriptyline might be able to inhibit the apoptosis that is characteristic for acute liver failure in Wilson disease [4]. However, none of these interventions have been thoroughly investigated in patients yet.

38.1.2 Menkes Disease

▪ Clinical Presentation

Symptoms generally appear in male infants at the age of 2-3 months, when the neurodegeneration provoked by the disease becomes manifest with seizures and hypotonia [16]. Sometimes, nonspecific signs can be present at birth, including prematurity, large cephalhaematomas, skin laxicity and hypothermia, which are often not recognised as Menkes disease at that time. The hair, if present, can already exhibit the characteristic pili torti, which will appear later on in all cases. Patients lose earlier developmental milestones and, progressively, hypotonia is replaced by spasticity. A typical facial appearance, with sagging cheeks and frontal bossing, gradually becomes prominent. Feeding difficulties, vomiting and/or chronic diarrhoea are common, and weight gain is generally insufficient; nevertheless, 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.

Besides the more prevalent, severe Menkes phenotype, less severe forms occur in 10-15% of the patients, with the *occipital horn syndrome* being the mildest. This syndrome is characterised by connective tissue abnormalities with minimal effects on neurodevelopment [17]. Bone disease with demineralisation, deformities and exostoses, particularly at the occipital insertion of the paraspinal muscles (hence its name), are characteristic. Furthermore, patients have urinary tract diverticuli, orthostatic hypotension and chronic diarrhoea. Skin and joint laxicity are common, but pili torti are rarely seen.

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 [18]. Patients have missense mutations in *ATP7A*, resulting in a mild reduction of the copper-transport capabilities of the corresponding protein, ATP7A. Obviously motor neurons are sensitive to minor perturbations in copper homeostasis, which, however, take years to cause the symptoms described above, which can also be seen in acquired copper deficiency.

▪ Metabolic Derangement

In Menkes disease, cellular copper uptake is normal but copper cannot be exported from cells owing to a defect in the ATP7A protein [1]. Copper efflux from the intestinal cells is severely reduced, and insufficient copper reaches the circulation, passes the blood-brain barrier and is incorporated into the cuproenzymes. Among the affected 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. Deficient activity of these enzymes is probably responsible for a significant part of the cerebral pathology in Menkes disease. Dysfunction of Cu/Zn superoxide dismutase seems to be compensated for by an increased activity of manganese superoxide dismutase, and as such probably does not contribute much to the neurodegeneration. Other enzymes influenced by

copper deficiency are lysyloxidase, a critical enzyme in collagen crosslinking, and tyrosinase, which is necessary for melanin formation.

- **Genetics**

Menkes disease is a rare condition with an incidence of approximately 1:250,000 [16], and is inherited as an X-linked recessive trait. It is caused by mutations in the *ATP7A* gene, localised on chromosome Xq13.3, and expressed in all tissues, except liver. The mutation spectrum in Menkes disease is wide, with lesions throughout the gene, without predominant mutations. Seven patients have been reported with chromosome abnormalities, mostly X-autosome translocations, visible on cytogenetic examination [19]. Gross deletions in the gene, encompassing one or more exons, or even almost the whole coding sequence, are found in approximately 15% of the cases [19]. Many single base-pair changes or insertion/deletions of a few base pairs have been described. The vast majority of these mutations are predicted to introduce a premature stop codon, probably resulting in a truncated, nonfunctional protein. No straightforward genotype/phenotype correlations have been found so far, although most patients with the occipital horn syndrome have splice site mutations that potentially permit small amounts of *ATP7A* to be transcribed [20].

- **Diagnostic Tests**

A level of serum copper (<11 μmol/l) and serum ceruloplasmin (<200 mg/l) below the usual range supports the diagnosis, 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 for Menkes disease, especially the ratio between dopamine and norepinephrine and the ratio of dihydroxyphenylacetic acid to dihydroxyphenylglycol in plasma [21]. The copper retention characteristic of Menkes disease can be demonstrated by measuring the increased accumulation and reduced efflux of radiocopper in cultured fibroblasts [22]. The final diagnosis requires identification of the mutation.

Prenatal diagnosis is preferably done by mutation analysis. If the mutation is unknown DNA studies can still be informative using intragenic markers. Carrier detection too should be done 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**

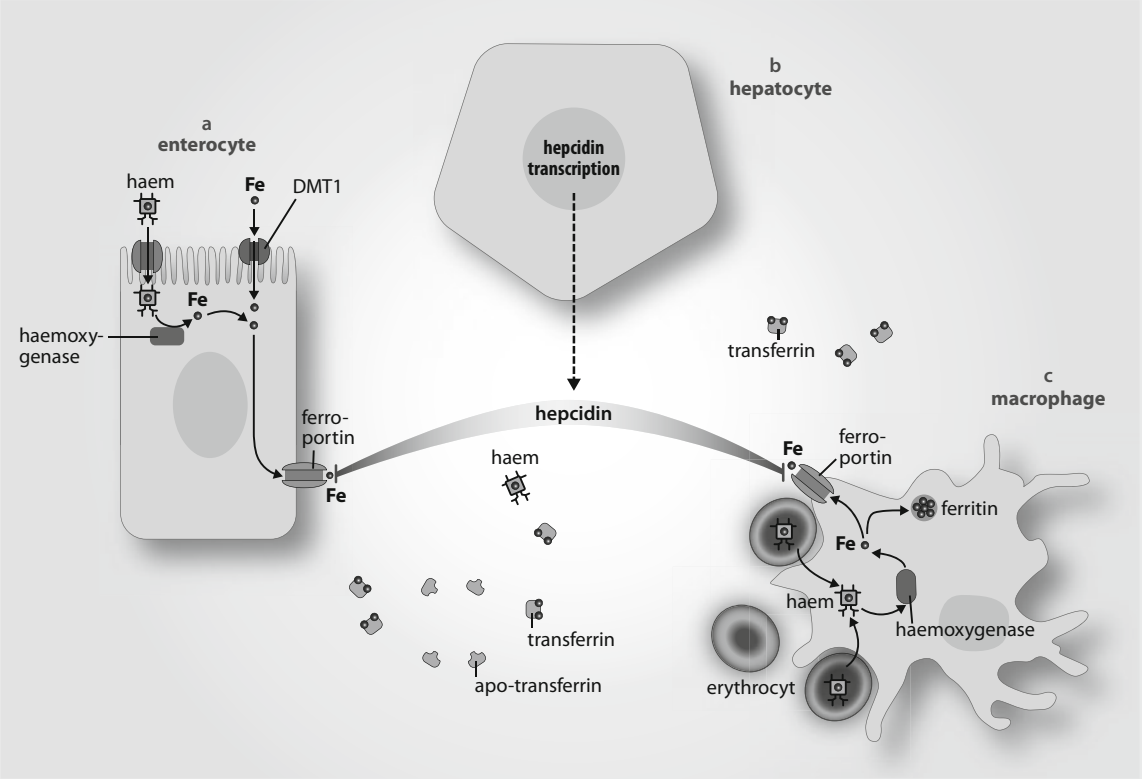
Classically, most patients die before 3 years of age due to infections or vascular complications, although with current medical care (improved feeding techniques) longer survival is not uncommon. However, parenteral treatment with copper histidine, the physiological copper complex in humans, can theoretically 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 [21]. When treatment is started early, i.e. in the first weeks of life, 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 ATP7B protein is present [21] or in those rare patients with mutations selectively affecting copper export, without affecting the ability to incorporate copper into cuproenzymes [23].

38.1.3 Other Copper Storage Disorders

Indian Childhood Cirrhosis (ICC), is characterised by a normal serum ceruloplasmin and extremely high liver copper levels (800-6,500 μg/g dry weight) [24]. 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, which is 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 [25]. 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 the water in certain wells. Given the similarities in clinical and biochemical characteristics it seems possible that all three entities are in fact one and the same disease. Since many of the patients are from consanguineous families, it is probable that an autosomal recessive mutation is responsible. *COMMD1* (formerly *MURR1)*, the gene mutated in the copper toxicosis seen in Bedlington terriers, has been excluded as a candidate gene [26]. No direct equivalent of this disease in humans has been described yet. However a mutation in CCS, the copper chaperone for superoxide dismutase, does indirectly lead to lowered intracellular concentration of the COMMD1 protein and causes liver disease in humans, as well as neurodevelopmental delay, epilepsy, congenital cataracts and a low serum copper [27].

Iron Metabolism

Synthesis of haem and other metalloproteins requires more than 20 mg of iron per day, which is mostly obtained by recycling: only 1-2 mg of iron is derived from intestinal absorption (◘ Fig. 38.2) primarily in the duodenum, through the divalent-metal transporter DMT1, which is encoded by the *SLC11A2* gene. Both in the enterocytes and in the macrophages, iron can also be acquired through direct uptake from erythrocytic haem, from which it is released by haemoxygenase. As iron is essential, but also toxic, its homeostasis is strictly regulated, as iron cannot be actively secreted from the body. Therefore, the export into the circulation, from both enterocytes and macrophages, by ferroportin encoded by the *SLC40A1* gene is tightly controlled by hepcidin, which is encoded by the *HAMP* gene. Several proteins, e.g. HFE encoded by the *HFE* gene, haemojuvelin encoded by the *HJV* gene and transferrin receptor 2 encoded by the *Tfr2* gene, regulate its synthesis. Hepcidin when high inhibits iron release by ferroportin and when low facilitates iron export into the circulation. In several cell types, including macrophages, iron can be stored bound to ferritin until needed. In the circulation, iron, which unbound is toxic, is bound to apo-transferrin, forming transferrin. Ceruloplasmin, a ferroxidase, also mediates efficient cellular iron export. Additional genes which, when mutated, affect particularly brain iron metabolism are listed below.



◘ **Fig. 38.2.** Uptake and release of iron by enterocytes and macrophages

Iron overload gives rise to haemochromatosis which, when fully developed, manifests with a triad of cirrhosis, diabetes and cardiomyopathy. The *archetypal hereditary haemochromatosis (type 1)* is caused by mutations in the HFE gene, 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 the HJV or in the HAMP gene, 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 the Tfr2 gene, symptoms are virtually identical

to those of type 1 haemochromatosis. While the first three subtypes are autosomal recessively inherited, *ferroportin-related hereditary haemochromatosis (type 4)* is autosomal dominant, caused by mutations in the *SLC40A1* gene, which mainly hamper copper export from macrophages. Patients have systemic iron overload, but mild microcytic anaemia might also be present. *Neonatal haemochromatosis*, once thought to be genetic, is now known to be a maternal alloimmune reaction to the infant liver, resulting in neonatal liver failure and iron overload in both the liver and extrahepatic organs.

Four disorders are known that provoke iron accumulation in the brain and neurodegeneration. *Pantothenate kinase deficiency*, caused by mutations in the PANK2 gene, and *infantile neuroaxonal dystrophy*, caused by mutations in the *PLA2G6* gene, both result in iron deposits in the basal ganglia, causing severe extrapyramidal symptoms, which generally become manifest in infancy or childhood. *Aceruloplasminaemia* is caused by mutations in the *CP* gene. 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 the *FTL* gene, resulting in deposition of iron and ferritin in the basal ganglia. Patients with *idiopathic neurodegeneration with brain iron accumulation* are a heterogeneous group, presenting with extrapyramidal symptoms due to iron accumulation in the basal ganglia, but without mutations in any of the aforementioned genes.

Iron-refractory iron deficiency anaemia (IRIDA) is caused by mutations in the *TMPRSS6* gene. 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 the *SLC11A2* gene, hampering the uptake of iron by erythroblasts and resulting in microcytic anaemia.

38.2 Iron

38.2.1 Systemic Iron Overload Syndromes (Haemochromatosis)

■ Classic Hereditary Haemochromatosis (Type 1)

■■ Clinical Presentation

Classic hereditary haemochromatosis, also called type 1 or HFE-related haemochromatosis, is an autosomal recessive disorder characterised by a slow but progressive accumulation of iron in various organs, which becomes clinically apparent during the 4th or 5th decade of life [28, 29]. 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, hypogonadotrophic hypogonadism, arthropathy and skin pigmentation are now seen only rarely [28, 29].

■■ 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 [28,31]. Other mutations in *HFE* are also described, e.g. H63D, with compound heterozygosity for H63D and C282Y being associated with iron overload [28, 29].

■■ 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 the *HFE* gene should be performed [28, 29].

■■ Treatment and Prognosis

At least half of all male and female C282Y homozygotes have normal serum ferritin levels and may never require therapy [28, 29]. In addition, many more will only have moderately elevated serum ferritin levels (200-1,000 ng/ml), and it is unclear at present whether all should have regular phlebotomies to reduce systemic iron load [31]. However, with serum ferritin levels exceeding 1,000 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 [28, 29, 31].

■ 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 in this subtype. Patients present in the 2nd and 3rd decades, mostly with hypogonadism and cardiomyopathy as a result of iron overload. Type

2A results from mutations in the *HJV* gene encoding for haemojuvelin, 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 [28, 29].

- **_TfR2_-related Hereditary Haemochromatosis (Type 3)**

The transferrin receptor 2 gene (*TfR2*) and its product, TfR2, are probably necessary for sensing iron levels and regulating hepcidin synthesis. Mutations in this gene result in an iron overload phenotype which resembles classic, HFE-related haemochromatosis, although patients are generally somewhat younger [28]. Elevated transferrin iron saturation 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 the *SLC40A1* gene, encoding ferroportin, which is not only expressed at the enterocyte, but also at the cellular membrane of the macrophages. The most common mutations (M-type disease) cause a loss of function, so that iron cannot be adequately exported from the macrophages to erythrocytic precursors, giving a combination of mild microcytic anaemia with low transferrin saturation, yet with a systemic iron overload and thus high ferritin concentrations. In this subtype tolerance to phlebotomy is limited by the concurrent anaemia. In addition, mutations have been identified in which ferroportin retains iron export capabilites but is resistant to hepcidin. These patients present with a more classic hepatic iron overload haemochromatosis phenotype [28, 32]

- **Neonatal Haemochromatosis**

Although now known to be an acquired disorder, until recently neonatal haemochromatosis (NH) was thought to be passed on by an autosomal recessive mode of inheritance. 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 mishandling of iron, giving severe siderosis of both liver and extrahepatic organs. This 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 [33]. 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 [34].

38.2.2 Neurodegeneration with Brain Iron Accumulation (NBIA)

- **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 progressive over the years, with involvement of the corticospinal tract and development of spasticity. Affected children lose the ability to walk within 10-15 years [35]. 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 the *PANK2* gene encoding pantothenate kinase 2, which is a key enzyme in the biosynthesis of co-enzyme A [35, 36]. 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.

- **Infantile Neuroaxonal Dystrophy (INAD)**

INAD is an autosomal recessive disorder caused by mutations in the *PLA2G6* gene encoding the calcium independent phospholipase enzyme iPLA2-VI, which catalyses the hydrolysis of glycerophospholipids. 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 [36, 37] (► Chapter 37).

- **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 [38, 39]. Clinically the disease consists in adult-

onset neurological disease (chorea, cerebellar ataxia, dystonia, parkinsonism and psychiatric signs), retinal degeneration and diabetes mellitus. The iron accumulation is due to the absence of ceruloplasmin, which, through its ferroxidase activity, is necessary for iron export, especially in the brain, where it is anchored to glycosylphosphatidylinositol. More than 30 aceruloplasminemia-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 (850-4,000 ng/ml). Desferrioxamine, a high-affinity iron chelator, reduces body iron stores and may therefore ameliorate diabetes as well as hepatic and neurological symptoms [40].

- **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. Patients present at adolescence and up to adult age with chorea, ataxia, rigidity and dystonia, as well as mixed complaints of cognitive dysfunction. So far six mutations have been described in the *FTL* gene in patients with this disease, all changing 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 [41].

- **Idiopathic Neurodegeneration with Brain Iron Accumulation**

Some patients will have neurodegeneration and extrapyramidal symptoms with accumulation of iron in the brain, yet be negative for mutations in any of the genes mentioned (*PANK2*, *PLA2G6*, *FTL*, *CP*). Some have onset in childhood, others in adulthood, while the rate of progression varies widely. They probably represent a heterogeneous group of patients, with – still unknown – genetic causes responsible for the pathological changes in a substantial proportion [36] (see also ► Chapter 37).

38.2.3 Iron Deficiency Syndromes

- **Iron-refractory Iron Deficiency Anaemia (IRIDA)**

This disease is caused by a deficiency of matriptase-2, which is encoded by the *trans*-membrane protease serine 6 (*TMPRSS6*) gene. If a mutation in both copies of this gene is present the normal cleavage of haemojuvelin is interrupted, resulting in high hepcidin levels [28]. This will result in iron deficiency, low transferrin saturation (<10%) and microcytic anaemia at a young age [42]. Oral iron supplementation is not effective, as high hepcidin levels will prevent iron release from the enterocytes, necessitating intravenous iron therapy.

- **DMT1 Deficiency**

So far mutations in the divalent-metal transporter 1 (DMT1) leading to microcytic anaemia have been reported in three patients [43]. DMT1, encoded by the *SLC11A2* gene, is present in the enterocyte, but also in erythroid precursors. It is at this site that DMT1 deficiency, which is autosomally recessively inherited, will give problems, as the erythroblasts cannot be properly loaded with iron. Deficient uptake of iron at the enterocyte can probably be bypassed by direct uptake of haem. 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 probably be avoided while haemoglobin is still maintained at an acceptable level [43].

38.3 Magnesium

Magnesium Metabolism

Magnesium is the second most abundant intracellular cation and plays an essential role in many biochemical processes as well as in neuromuscular excitability. A 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 proceeds mainly through active uptake by the *TRPM6* magnesium channel, which is encoded by the *TRPM6* gene and 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. Disturbance of tubular handling of magnesium through mutations in the *CLDN16*, *CLDN19*, *FXYD2* or *EGF* gene will give a specific phenotype in each case, which includes hypomagnesaemia.

Primary hypomagnesaemia with secondary hypocalcaemia generally presents in the first months of life with

increased neuromuscular irritability or even frank convulsions. It is caused by mutations in the *TRPM6* gene, reducing uptake of magnesium from the gut. *Hypomagnesaemia with hypercalciuria and nephrocalcinosis* can be caused by mutations in the *CLDN16* or in the *CLDN19* gene, 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. *Isolated dominant hypomagnesaemia* results from mutations in the *FXYD2* gene and can cause generalised convulsions. Isolated *autosomal recessive hypomagnesaemia* results from mutations in the EGF gene and is asymptomatic.

38.3.1 Primary Hypomagnesaemia with Secondary Hypocalcaemia

▪ Clinical Presentation

Primary hypomagnesaemia with secondary hypocalcaemia (HSH) is a rare autosomal recessive disorder. It was first recognised in 1965, and since then more than 70 infants from all over the world have been described [44]. Patients commonly present in the first months of life with generalised 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 [45]. A lowered renal threshold for magnesium, causing a significant renal magnesium leak, is a contributing factor and also generally prevents serum magnesium from completely normalising during supplementation [46]. The disease is caused by a defect of the TRPM6 protein, a member of the long transient receptor potential channel (TRPM) family, which complexes with its closest homologue, TRPM7, to form an ion-channel for magnesium at the cell surface. Genetic lesions of TRPM6 prevent assembly of this complex and hence impair magnesium transport [47].

Severe hypomagnesaemia blocks synthesis and/or release of parathormone. In addition, when hypomagnesaemia is present, the administration of parathormone (PTH) fails to induce a rise in serum calcium. The hypocalcaemia in HSH is thus secondary to low parathormone levels in combination with some form of end-organ resistance.

▪ Genetics

Primary hypomagnesaemia is an autosomal recessive disorder that is caused by mutations in the *TRPM6* gene [48, 49]. This gene is expressed in the small and large intestine as well as in the cells lining the distal tubules. To date over 20 mutations have been identified [46].

▪ Diagnostic Tests

Primary hypomagnesaemia is characterised by 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 hypomagnesaemia the urinary excretion of magnesium is reduced and PTH levels are inappropriately low. No evidence of 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 stabilisation, 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) [44, 46]. With this regimen, serum calcium normalises, but serum magnesium will generally remain just below normal [44, 46]. Dividing oral magnesium supplementation into three to five doses will reduce fluctuations of serum magnesium and will prevent the development of chronic diarrhoea in many, but not in 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 of developing psychomotor retardation.

38.3.2 Hypomagnesaemia with Hypercalciuria and Nephrocalcinosis

▪ Clinical Presentation

Over 80 patients with familial hypomagnesaemia with hypercalciuria and nephrocalcinosis (FHHNC) have been reported [50, 51]. Patients usually present during childhood with recurrent urinary tract infections, polyuria/polydipsia and/or haematuria. At presentation, renal stones are seen in 13-25% of patients, while nephrocalcinosis, rare at presentation, will ultimately develop in all. Clinical signs of hypomagnesaemia, such as seizures, are less common, in line with only moderately depressed serum magnesium levels.

▪ Metabolic Derangement

In most patients FHHNC is caused by a defect of claudin-16 (formerly paracellin-1), while in a subset of patients claudin-19 is defective [51, 52]. In the thick ascending limb of Henle's loop these two proteins form a complex at the tight junction [52]. Mutations in either component of this complex will change ion selectivity at the tight junction, influencing the transepithelial diffusion potential, the driving force of magnesium reabsorption [53]. Disturbance of this process leads to renal loss of magnesium and calcium, with secondary development of nephrocalcinosis and ultimately renal failure.

▪ Genetics

The genes encoding claudin-16, *CLDN16* (formerly paracellin-1, *PCLN-1*), and claudin 19, *CLDN19*, belong to the claudin multigene family [53]. In FHHNC patients, to date over 20 distinct mutations in *CLDN16* have been identified, and 3 mutations in *CLDN19,* all single base-pair changes [51, 52]. The renal phenotype does not differ between the two genotypes. However, while some patients with *CLDN16* mutations have mild ocular pathology (strabismus, myopia), all patients with *CLDN19* mutations have severe visual impairment [52]. This is consistent with the high *CLDN19* expression at the retina.

▪ Diagnostic Tests

Serum magnesium is low (mean 0.40 mmol/l, range 0.23-0.61 mmol/l) [51], but less so than in primary hypomagnesaemia. Median calcium excretion is 10 mg/kg/24 h (normal 4-6 mg/kg/24 h). Serum calcium is somewhat below the lower level of normal in about half of the patients. Other biochemical abnormalities include hypocitraturia and mild hyperuricaemia. At diagnosis, the glomerular filtration rate is already reduced in the majority of patients, and it 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 influence the progression of renal failure significantly. In a series of 33 patients, all showed a deterioration in glomerular filtration rate, and one third developed end-stage renal disease during adolescence [51]. The median age at end-stage renal disease in this group was 14.5 years (range 5.5-37.5 years).

38.3.3 Isolated Dominant Hypomagnesaemia

Autosomal dominant hypomagnesaemia is caused by a reduced tubular threshold for magnesium and is associated with a lowered urinary calcium excretion [54]. Patients have hypomagnesaemia (0.40 mmol/l; normal 0.65-1.20 mmol/l) and may display generalised convulsions, although most do not have any symptoms.

The disorder is caused by mutations in the *FXYD2* gene, which encodes the γ-subunit of Na^+K^+-ATPase, which is expressed in the distal tubules [55]. Normal function of the Na^+K^+-ATPase is necessary for adequate renal magnesium handling, and the mutation identified in the γ-subunit specifically changes its activity, accounting for the dominant negative effect of the mutation. The disorder seems genetically heterogeneous, as an American family with a similar phenotype has been described which does not map to the 11q23 region containing the *FXYD2* gene [56].

38.3.4 Isolated Autosomal Recessive Hypomagnesaemia

Isolated autosomal recessive hypomagnesaemia has been described in two children from a consanguineous family [57]. 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 the *EGF* gene, leading to insufficient activation of kidney TRPM6, which is essential for renal tubular magnesium reabsorption [58].

38.4 Manganese

Manganese Metabolism

Manganese is an essential trace element and a cofactor for enzymes involved in peptide and amino acid metabolism (prolidase, arginase, glutamine synthetase), protein *O*-glycosylation, carbohydrate metabolism, superoxide elimination and others. As manganese is also potentially toxic, its concentration must be carefully controlled. However, this process is not well understood at present, although it is known that DMT1 (divalent-metal transporter 1), which is involved in iron uptake and transport, probably has a similar function for manganese. In addition, several intracellular trans-

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porters are known to be involved in both manganese and calcium transport, i.e. the Golgi-associated secretory pathway (SPCA) and the sarco- /endoplasmatic reticulum (SERCA) Ca2+ ATPases, but the exact role of these transporters in manganese metabolism is not yet clear.

Isolated autosomal recessive hypermanganesaemia presents with manganese deposits in the basal ganglia and an extrapyramidal motor disorder in combination with liver cirrhosis and polycythaemia.

Calcium/manganese transporter defects are found in two autosomal dominant blistering skin disorders characterised by acantholysis and dyskeratosis of the epidermis (Hailey-Hailey disease and Darier-White disease) and in a muscle disorder (Brodier disease).

38.4.1 Isolated Autosomal Recessive Hypermanganesaemia

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 can escape normal control mechanisms, such as exposure to manganese dust in adults working in the mining or welding industries, or in patients on prolonged TPN, especially neonates [59, 60]. 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.

Recently three patients were described with hypermanganesaemia not related to environmental exposure or TPN [61, 62]. These patients presented with high blood manganese levels (>3000 nmol/l), manganese deposits in the basal ganglia and an extrapyramidal motor disorder in combination with liver cirrhosis and polycythaemia. As two of these patients were born to consanguineous parents autosomal recessive inheritance is probable [61]. In the only patient still living in this family, treatment of the manganese overload was successfully achieved by a combination of chelation with disodium calcium EDTA and iron supplementation. Iron competively inhibits manganese uptake in the intestine via DMT1.

38.4.2 Disorders Affecting Calcium/ Manganese Transporters

Manganese can be transported by a number of transporters that also transport calcium, including the Golgi-associated secretory pathway (SPCA) and sarco-/endo-plasmatic reticulum (SERCA) ATPases [61]. An SPCA ATPase gene (*ATP2C1*) is mutated in Hailey-Hailey disease, an autosomal dominant blistering skin disorder characterised by acantholysis and dyskeratosis of the epidermis. A SERCA ATPase gene (*ATP2A2*) is mutated in another autosomal dominant disorder, causing skin disease characterised by acantholysis and dyskeratosis (Darier-White disease or keratosis follicularis). Another SERCA ATPase gene (*ATP2A1*) is mutated in Brody disease, a disorder of skeletal muscle relaxation following contraction. The role (if any) of defective manganese transport in the pathogenesis of these disorders is uncertain.

38.5 Selenium

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 the *SECISBP2* gene 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 the *SepSecS* gene.

Two rare inborn errors of metabolism are identified within this system. The first, involving mutations in the *SECISBP2* gene, 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 [63, 64]. The second inborn error involves mutations in the *SepSecS* gene, giving progressive microcephaly, profound mental retardation and severe spasticity, an autosomal recessive clinical entity known as progressive cerebello-cerebral atrophy (PCCA) [65].

38.6 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 α_2-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 the intracellular zinc concentration by promoting zinc influx into cells (or the release of zinc from intracellular vesicles).

Acrodermatitis enteropathica is due to mutations in the *SLC39A4* gene 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. *Zinc deficiency in breastfed babies* presents with the same dermatological symptoms as acrodermatitis enteropathica. It is caused by heterozygous mutations in the *SLC30A2* gene. *Hyperzincaemia with hypercalprotectinaemia* is characterised by extremely elevated levels of calprotectin thought to cause uncontrolled, harmful inflammatory reactions. *Autosomal dominant hyperzincaemia without symptoms* is most probably a non-disease.

38.6.1 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 localised at acral and periorificial sites [66, 67]. At onset, these skin lesions are erythematous, while after the 1st 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, which are 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 seem 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 a partial block in the intestinal absorption of zinc, as demonstrated in vivo by oral application of ^{65}Zn [68]. Similarly, zinc absorption in intestinal biopsies of patients is reduced [69]. This defect is due to dysfunction of the protein involved in AE (ZIP4). The insufficient zinc absorption results in severe 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 the *SLC39A4* gene localised on chromosome 8q24.3 [70, 71] *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 30 mutations have been identified so far, mainly in families from Europe, the Middle East and North Africa [72].

■ Diagnostic Tests

Serum zinc levels are usually low (7.1±5.0 μmol/l; normal 11.9-19.4 μmol/l), although normal values are found in at least 15% of patients [67]. 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, hypobetalipoproteinaemia and reduction of serum vitamin A. In many patients, both humoral and cell-mediated immunity are depressed [73]. 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 with radiolabelled zinc [68]. However, a more practical approach is to start zinc therapy when the clinical diagnosis is suspected and await the response, which should occur within 1 week. If the clinical signs of AE are equivocal zinc therapy can be stopped 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 the *SLC39A4* gene for mutations is theoretically superior. However, this test is only available in a few centres, and it can take several months to obtain results.

- **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 [74] and is now used in all patients. The usual dose is 150-400 mg zinc sulfate/day (equivalent to 35-90 mg elemental zinc/day), with 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 sulfate/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. The prognosis with treatment is excellent.

38.6.2 Zinc Deficiency in Breastfed Babies

Rarely, zinc deficiency with acrodermatitis can occur in breastfed babies, especially in premature infants, as they have an increased zinc requirement in combination with a reduced capacity for zinc uptake in the gut [75]. 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 the *SLC30A2* gene [76].

38.6.3 Hyperzincaemia with Hypercalprotectinaemia

A syndrome with extreme elevation of plasma zinc (77-200 μmol/l), recurrent infections, hepatosplenomegaly, arthritis, anaemia and persistently raised concentrations of C-reactive protein has recently been described [77, 78]. Levels of serum calprotectin, the major zinc-binding protein of phagocytes, are more than 1,000 times the upper limit of normal. It is speculated that the very high concentration of this protein results in the uncontrolled and harmful inflammatory reactions which characterise this syndrome, while the hyperzincaemia is caused by the zinc-capturing properties of calprotectin. Treatment with cyclosporin A or tacrolimus has been tried, but with varying results [78]. Inheritance of this syndrome is not clear yet.

38.6.4 Autosomal Dominant Hyperzincaemia Without Symptoms

Elevated serum zinc (40-70 μmol/l) was described in seven family members from one large pedigree [79]. The condition seems to be inherited in an autosomal dominant fashion. Zinc concentrations in hair and erythocytes were normal in these patients, as was serum albumin, to which most of the excess zinc seemed to be bound. There were no clinical symptoms and no additional biochemical abnormalities, so this condition appears to be benign.

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X Organelle-Related Disorders: Lysosomes, Peroxisomes, and Golgi and Pre-Golgi Systems

Disorders of Sphingolipid Metabolism and Neuronal Ceroid-Lipofuscinoses

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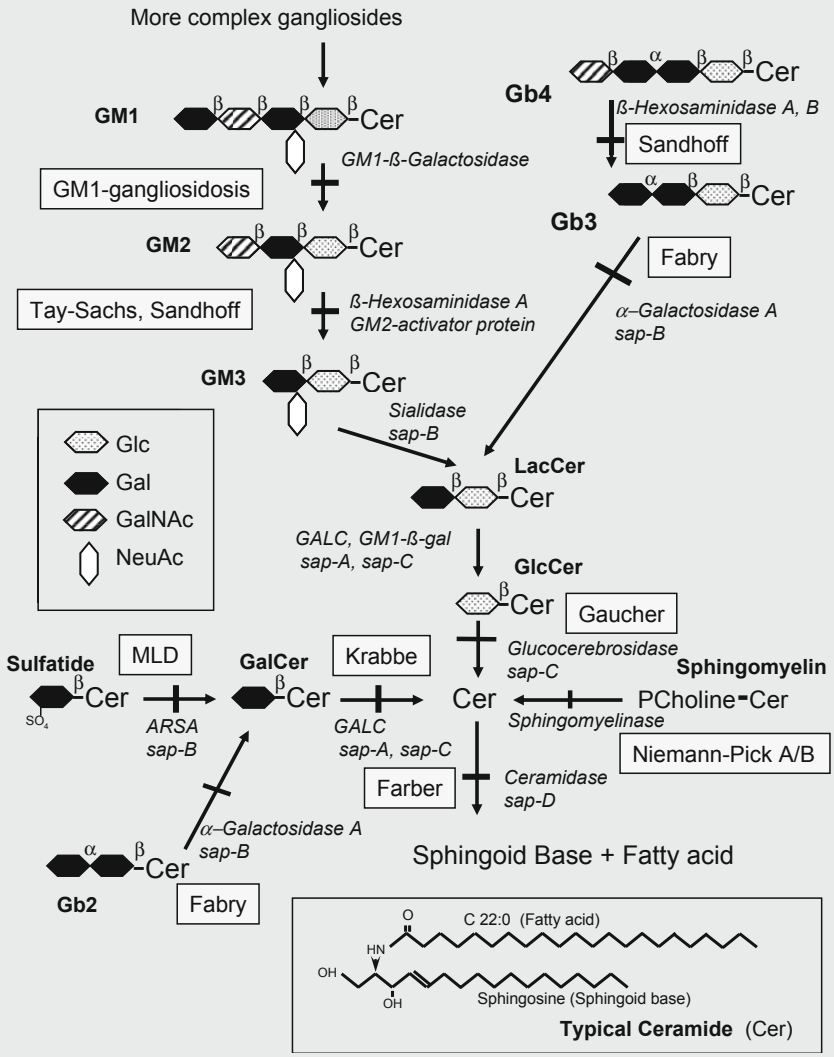
Sphingolipid Structure and Metabolism

Sphingolipids are derived from the lipophilic compound ceramide (Cer) (◘ Fig. 39.1). Ceramides are composed of a variable long-chain amino alcohol sphingoid base (sphingosine being the prototype), which is attached by an amide linkage to a variable long- or very-long-chain fatty acid. 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) or phosphorylethanolamine. Glycosphingolipids contain one (galactose or glucose) or several sugar residues, and can be very complex. Sialic acid-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) are lacking the



◘ **Fig. 39.1.** Sphingolipid structure and degradation. *ASA*, arylsulfatase A; *Gal*, galactose; *GALC*, galactocerebrosidase; *GalCer*, galactosylceramide (or galactocerebroside); *GalNAc*, *N*-acetylgalactosamine; *Gb2*, galabiosylceramide; *Gb3*, globotriaosylceramide; *Gb4*, globotetraosylceramide (globoside); *Glc*, glucose; *GlcCer*, glucosylceramide (or glucocerebroside); *GM1*, GM1 ganglioside; *GM2*, GM2 ganglioside; *GM3*, GM3 ganglioside; *LacCer*, lactosylceramide; *MLD*, metachromatic leukodystrophy; *NeuAc*, *N*-acetylneuraminic acid (sialic acid); *Pcholine*, phosphorylcholine; *sap*, saposin. Enzyme defects are indicated by *solid bars* across the *arrows*

fatty acid of ceramide. Only the main sphingolipids implicated in sphingolipidoses are depicted in ◘ Fig. 39.1.

Sphingolipids are synthesised and degraded in different cellular compartments. Sphingolipids (except galactosylceramide) are formed in the Golgi apparatus, glycosphingolipids by sequential addition of monosaccharides to ceramide, catalysed by specific glycosyltransferases. Thereafter they are transported and inserted in the plasma membrane where they play a structural and functional role. Their degradation (◘ Fig. 39.1), after transport by the endosomal pathway to the lysosome, proceeds by stepwise hydrolysis by specific sphingohydrolases, some of which may need co-factors called sphingolipid activator proteins 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 involved in their degradative pathway. Traditionally, this subgroup also includes Niemann-Pick C disease, which is characterised by impaired cellular trafficking of lipids. These diseases may have visceral, neurovisceral or purely neurological manifestations. The neuronal ceroid-lipofuscinoses (NCLs) constitute another group of lysosomal disorders, with accumulation of autofluorescent ceroid lipopigments and a severe neurodegenerative course including retinopathy, epilepsy, motor abnormalities and dementia. 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, but also fetal presentations have been overlooked in the past. No overall screening procedure is available to date. In most sphingolipidoses and in three of the NCLs the diagnosis is made by demonstration of the enzymatic defect, generally expressed in most cells and tissues (leukocytes represent the most widely used enzyme source). In the remaining diseases, more complex biochemical tests or a molecular genetic assessment are necessary. For most NCLs, electron microscopy remains crucial as an orientation test. Specific therapies are well established for non-neuronopathic Gaucher and Fabry diseases, and emerging for some others, including Niemann-Pick type C. In spite of active research, for the neurological forms of sphingolipidoses and the ceroid lipofuscinoses, knowledge on pathophysiology and progress towards therapy remain limited.

39.1 Gaucher Disease

39.1.1 Clinical Presentation

Historically, three clinical phenotypes are recognised, but the full disease spectrum is actually 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 [1].

Type I, defined by the lack of neurological symptoms, accounts for about 90% of all cases. Most commonly diagnosed in adults, it can present at any age [2]. There is a wide variability in the pattern and severity of the symptoms, from extremely handicapping to asymptomatic forms, with most symptomatic patients having visceral, haematological and (more frequently in adults) skeletal disease [3]. Children often show severe splenomegaly, generally associated with hepatomegaly, but 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 often 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. A puzzling association between mutations of the *GBA* (acid ß-glucosidase) gene and parkinsonism has been well documented [4, 5].

Type II (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 [6-8]. 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) [6-8].

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 [9, 10].

Type III (Subacute or chronic neuronopathic Gaucher disease) is heterogeneous. Division into subtypes is now considered artificial [8]. 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 [11]. 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 [8, 11]. 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.

39.1.2 Metabolic Derangement

The primary metabolic defect resides in a block of the lysosomal degradation of glucosylceramide (glucocerebroside) and glucosylsphingosine. In the vast majority of cases this is due to a deficiency of acid β-glucosidase (glucocerebrosidase, glucosylceramidase). Exceedingly rare cases, presenting as type III [12] or I [13] are due to a deficiency of the saposin (SAP) *sap*-C, which is required for the in vivo hydrolysis of glucosylceramide. Glucosylceramide (glucocerebroside) accumulates massively in liver and spleen of patients in all types. Although elevated in cerebral grey matter of type II and type III patients, its concentration in brain remains low. Pathophysiology of the disease is poorly understood [1]. Glucosylsphingosine, a highly cytotoxic compound, Ca^{2+}, and inflammatory responses seem to be involved.

39.1.3 Genetics

The disease (except for *sap*-C deficiency) is caused by mutations of the *GBA* (acid β-glucosidase) gene (1q21) [1]. More than 330 mutations are known. N370S, the most common mutation in Ashkenazim, is also very frequent in Caucasian populations. Even as a genetic compound with another mutant allele, N370S is always associated with a non-neuronopathic phenotype. The severity can vary widely in Gaucher patients with the same genotype, including N370S homozygotes [14-16]. The second most frequent mutation, L444P, first described in Norbottnian type III, is more frequently associated with types II and III. Complex alleles due to genetic rearrangements are more often associated with severe forms, including perinatal lethal forms [10].

39.1.4 Diagnostic Tests

Bone marrow examination may reveal Gaucher cells, often multinucleated reticuloendothelial cells with a vacuolated cytoplasm, with a »wrinkled tissue paper« appearance. In serum, levels of chitotriosidase, angiotensin-converting enzyme, tartrate resistant acid phosphatase and the chemokine CCL18/PARK are typically very elevated. These markers are used to monitor treated patients (► below). The demonstration of a deficient glucocerebrosidase activity in lymphocytes (preferably) or leukocytes can be done using an artificial fluorogenic substrate. An assay on dry blood spots using a more specific substrate and tandem mass spectrometry has been developed [17]. Cultured cells have a much higher activity. DNA testing, required for carrier detection, has also improved diagnostic accuracy for patients with high levels of enzyme activity. Studies of lipids in liver and/or spleen might allow a retrospective diagnosis in autopsy material. Frozen tissue is optimal, but sphingolipid analysis is possible on formalin-fixed tissues. In *sap*-C deficiency, glucocerebrosidase activity is normal, but the findings of Gaucher cells on a bone marrow smear and strikingly elevated chitotriosidase levels should lead to molecular analysis of the *PSAP* gene [13]. Study of the lipid profile in a liver biopsy would also demonstrate pathognomonic glucosylceramide storage [12].

39.1.5 Treatment and Prognosis

Two approaches are currently available for the specific treatment of type I (and to some extent type III [8]) patients: enzyme replacement therapy (ERT) and substrate reduction therapy (SRT). Splenectomy enhances the risk of progression of the disease at other sites, especially bone and lung, and can generally be avoided by the institution of ERT. Pregnancy is not contraindicated in untreated patients, although bleeding may become critical before

and after birth. There is now a good experience of ERT throughout pregnancy. Enzyme therapy, conducted with slow infusions of a recombinant enzyme modified to expose mannose groups, thus ensuring optimal uptake by macrophages, has largely proved safe and effective [1]. Imiglucerase has been used worldwide for 15 years [18], and two other products, velaglucerase alfa [19] and taliglucerase alfa, are coming onto the market. The natural history of type I 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 II. 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. Partial inhibition of glucosylceramide synthase can be achieved by small molecules that can be administered orally. Experience in the use of the iminosugar miglustat for treatment of mild forms of Gaucher type 1 has been gained in recent years [1, 20]. Very recently, clinical trials using a ceramide-like inhibitor, eliglustat, have been reported [21]. Ongoing clinical trials using the chaperone isofagomine [20] have been halted.

39.2 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 clearly separate entities, based on their metabolic defect: sphingomyelinase deficiencies, including the historical types A and B, and lipid trafficking defects, corresponding to Niemann-Pick disease type C (caused by deficiency neither of a lysosomal enzyme nor of its co-factor (► below). Considering that Niemann-Pick type C is now established under that name, the collective term of »acid sphingomyelinase-deficient Niemann-Pick disease« (ASM-deficient NPD) or »ASM deficiency« has been proposed for primary sphingomyelinoses (i.e. types A and B, and intermediate forms) [22].

39.2.1 Clinical Presentation

Sphingomyelinase 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 [23]. 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. Slowed nerve conduction velocity 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 [23].

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 [24], but discovery may occur at any point from birth until late adulthood. Bruising and epistaxis are frequent. Hypersplenism occurs in a small proportion of patients. Splenectomy is seldom necessary, and 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 [25]. 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 [24]. 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 [24]. 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 [26, 27]. Some others are closer to type B, with minimal nervous system involvement (often peripheral neuropathy) and/or mild mental retardation [28].

39.2.2 Metabolic Derangement

A primary deficiency of the lysosomal (or acid) sphingomyelinase resulting from mutations on the *SMPD1* gene leads to the progressive accumulation of sphingomyelin in systemic organs in all types of the disease, and in brain in the neuronopathic forms [22]. Sphingomyelin storage is massive in liver and spleen in type A, and slightly less so in type B. A significant increase of unesterified cholesterol occurs secondarily. By in vitro measurements using the natural substrate, a marked sphingomyelinase deficiency is observed in all patients. In situ hydrolysis of labelled sphingomyelin by living cultured fibroblasts demonstrates a significant level of residual activity in many type B patients, suggesting that the mutated enzyme has retained enough catalytic activity to limit accumulation and protect the brain. Sphingosylphosphorylcholine (increased in type A brain) may participate in the pathogenesis of the brain dysfunction.

39.2.3 Genetics

More than 130 disease-causing mutations of the *SMPD1* (acid sphingomyelinase) gene (11p15) are known [22, 29]. In Ashkenazi Jewish type A patients, 3 mutations (R496L, L302P, fsP330) account for >90% of alleles. R608del, 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 is associated with late-onset neurological involvement. The *SMPD1* gene appears to be paternally imprinted, with some heterozygous carriers showing signs of the disease because of preferential expression of the maternal allele owing to methylation of the paternal allele [30].

39.2.4 Diagnostic Tests

Bone marrow usually reveals the presence of (nonspecific) foamy histiocytes or sea-blue histiocytes. Chitotriosidase is moderately elevated. The diagnosis is made by demonstration of a deficiency in sphingomyelinase activity in leukocytes (or lymphocytes) or in cultured cells (which have a much higher level of activity). The choice of a specific substrate is critical. Sphingomyelin radioactively labelled on the choline moiety is the gold standard. A sensitive assay using a short-chain analogue and tandem mass spectometry has been published [17]. The fluorogenic substrate should be used with caution [27, 31]. The in vitro assay does not reliably distinguish A from B phenotypes. The more informative loading test in living fibroblasts is not performed routinely.

39.2.5 Treatment and Prognosis

No specific therapy is yet available. Experience of bone marrow transplantation (BMT) is limited, but has not appeared to improve symptoms in type A patients. In type B, splenectomy may have a deleterious effect on the lung disease. Most female type B patients enjoy uncomplicated pregnancies, although careful monitoring for bleeding is advisable. Preclinical trials using the human recombinant enzyme in a knockout mouse model provided the proof of principle for ERT for type B [32]. Results of a phase I clinical trial in type B patients have been published [33]. A phase II trial is planned for the near future. In the neuronopathic mouse model, several other experimental therapeutic measures have been tried with partial results, including intracerebral gene therapy using an AAV vector. Recent studies showing a beneficial effect of the heat-shock protein (HSP) 70 on lysosomes of sphingomyelinase-deficient fibroblasts led to the hypothesis that this molecular chaperone could potentially open up a new therapeutic approach [34].

39.3 GM1 Gangliosidosis

39.3.1 Clinical Presentation

First descriptions of infantile GM1 gangliosidosis by B.H. Landing and J.S. O'Brien 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 recognised later. Three clinical phenotypes are recognised [35].

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 expressions. 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 *fetal 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 [36, 37]. Cognitive impairment is absent to moderate, and there are no ocular abnormalities. Bone changes are inconstant. The course of the disease is very slow. Re-evaluation of Japanese adults with a spinocerebellar ataxia-like syndrome, progressive dementia and low intracellular β-galactosidase indicate that they may be affected by another disease entity, with secondary deficiency of β-galactosidase.

39.3.2 Metabolic Derangement

GM1 gangliosidosis is due to a deficiency of lysosomal acid β-galactosidase, which cleaves glycoconjugates containing a terminal β-galactosidic linkage and is necessary for the degradation not only of GM1 ganglioside and other glycosphingolipids, but also of galactose-containing oligosaccharides and keratan sulfates. As a consequence, 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/cathepsin A (PPCA) and N-acetylgalactosamine-6-sulfate sulfatase [38]. 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). 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 major 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 pathogenetic significance, have been reported. Galactose-containing oligosaccharides have been found in liver and urine. Keratan sulfate and other mucopolysaccharides accumulate in liver and spleen. The amount of keratan sulfate excretion in urine is smaller in GM1 gangliosidosis than in Morquio B disease.

39.3.3 Genetics

About 150 mutations of the gene for acid β-galactosidase, *GLB1* (3p21.33), have been described. Neither the type nor location of the mutation correlates well with a specific phenotype.

39.3.4 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, cranial 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. Oligosaccharide excretion can, however, 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 using an artificial chromogenic or fluorogenic substrate. A subsequent study of neuraminidase (in leukocytes or cultured fibroblasts) should be performed systematically in every β-galactosidase deficient patient to exclude galactosialidosis.

39.3.5 Treatment and Prognosis

No specific treatment is available to date. Substrate reduction therapy or chaperones are potential approaches for clinical trials in late-onset forms [20, 35].

39.4 GM2 Gangliosidoses

GM2 gangliosidoses are divided into three different 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 ganglioside GM2, 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 β-subunit and thus of hexosaminidase A (αβ-heterodimer), Sandhoff disease, to a deficiency of the β-subunit and thus of both hexosaminidase A and B (ββ-homodimer). Classic Tay-Sachs disease has a much higher incidence in the Ashkenazi Jewish population than in other ethnic groups. Infantile forms are by far the most common, but juvenile and adult forms are also recognised. A particular enzymatic variant of Tay-Sachs disease (the B1 variant) has a high incidence in Northern Portugal [39] and is globally more frequent in southern Europe. Variant AB is exceedingly rare (<10 reported cases), albeit probably underdiagnosed.

39.4.1 Clinical Presentation

The **infantile** forms of the three subtypes have a very similar presentation. 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 [40] are mostly due to a deficiency of 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 [41, 42]. Some patients show autonomic dysfunction.

39.4.2 Metabolic Derangement

The normal catabolism of GM2 ganglioside requires the GM2 activator protein first to bind to and extract GM2 from the plasma membrane before presenting it for cleavage to hexosaminidase A (the αβ-heterodimer). Hexo-

saminidase B, the ββ-homodimer, hydrolyses other substrates with a terminal hexosamine (glycoproteins and glycolipids), but not ganglioside GM2. In Tay-Sachs disease (affecting the α-subunit), hexosaminidase A only is deficient. In Sandhoff disease (affecting the β-subunit) both hexosaminidases are inactivated. 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. Apoptotic neuronal death and macrophage-/microglia-mediated inflammation have been suggested as possible mechanisms of the neurodegeneration process.

39.4.3 Genetics

More than 130 mutations of the *HEXA* gene (on chromosome 15) have been identified. Three mutations – an insertion (+TATC1278) and a splicing defect (IVS12 1421+1G>C) in infantile cases, a missense (G269S) in adult forms – account for 95% of the Ashkenazi Jewish alleles. Mutations at codon 178 altering the three-dimensional structure of the enzyme, such as R178H, result in a particular enzymatic variant or B1 variant presenting as a juvenile form in the homozygous state. A relatively good genotype-phenotype correlation has been reported. More than 40 mutations of the *HEXB* gene and 6 of the GM2 activator *GM2A* gene (both on chromosome 5) have been described.

39.4.4 Diagnostic Tests

In Tay-Sachs and Sandhoff disease, the clinical diagnosis can easily be confirmed by appropriate 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; to diagnose hexosaminidase A deficiencies, the direct assay 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* gene sequencing.

39.4.5 Treatment

Seizures are generally responsive to standard treatment. No effective curative treatment is currently available. A randomised, controlled phase II trial using miglustat [20] in patients with an advanced stage of adult-onset Tay-Sachs disease failed to document any therapeutic effect [43]. Neither was improvement found in a study of two patients with Tay-Sachs disease and three with the juvenile form of Sandhoff disease [44]. A trial involving pyrimethamine is ongoing [45].

39.5 Krabbe Disease

39.5.1 Clinical Presentation

Krabbe disease (or globoid cell leukodystrophy) leads to demyelination of the central and peripheral nervous system. Its estimated overall incidence is between 1 in 100,000 and 1 in 150,000 live births. It is more frequent in Scandinavia (but not in Finland). The classic infantile form accounts for about 80% of diagnosed cases. Later onset cases appear to be more common in southern Europe, especially Italy and Sicily. The incidence of adult-onset cases has been underestimated.

In the **infantile** form [46], the disease usually starts within the first 6 months after birth (sometimes before the age of 3 months). Initial symptoms include increasing irritability, crying, vomiting and other feeding problems, hyperesthesia, tonic spasms on light or noise stimulation, and signs of peripheral neuropathy. Episodic unexplained fever is 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.

Clinical diagnosis of late-onset Krabbe disease is much more difficult. The **late infantile** and **juvenile** forms start between the ages of 15 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, especially in the late infantile form [47]. 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 reported as infrequent, but 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 [48, 49] 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 mental deterioration. At MRI, hyperintensities along the pyramidal tracts are a characteristic and nearly constant sign.

39.5.2 Metabolic Derangement

Krabbe disease results from galactosylceramidase (or galactocerebrosidase, cerebroside β-galactosidase) deficiency, a lysosomal enzyme that catabolises galactosylceramide – a major lipid component of myelin – as well as lactosylceramide and galactosylsphingosine. In vivo, galactosylceramide degradation further requires the saposin (SAP) *sap*-A. Two cases due to *sap*-A deficiency are known [50]. Galactosylceramidase deficiency leads to an accumulation of galactosylceramide in the pathognomonic 'globoid cells' (multinuclear 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 especially, to underlie the early destruction of oligodendrocytes characteristic of the infantile form, and thus an arrest of myelin formation [51].

39.5.3 Genetics

The galactosylceramidase (*GALC*) gene is located on 14q31. At least 80 mutations are known. The most frequent mutant allele (>35% in most European countries and in the USA) associates a large (30-kb) deletion and a polymorphism and seems to originate from Sweden. T513M and Y551S are also frequent. G270D is common among adult-onset patients [52]. Some common polymorphisms (especially 1637G>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 [52]. Two unrelated infantile cases were assigned to a mutation in the sap-A domain of the *PSAP* gene.

39.5.4 Diagnostic Tests

Motor nerve conduction velocity is 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 [53]. In late-onset cases, T_2-weighted images may show more localised areas of hyperintensity with less involvement of cerebellum and deep grey matter [54, 55]. In adult-onset cases, typical T_2 hyperintensities along the pyramidal tracts involving optic radiations and corticospinal tracts are nearly constant. 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 [56]. 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 or cultured fibroblasts. This assay is not straightforward and is subject to several pitfalls. The use of a natural radiolabelled substrate is the accepted the gold standard. More recently, a C8-ß-D-galactosylceramide analogue has shown good specificity and high sensitivity when combined with final tandem mass spectrometric analysis [17]. This method, which can be applied to dry blood spots, is being used for neonatal screening in New York State [57]. Published experience is still limited regarding an alternative and less sensitive fluorogenic substrate. Prenatal diagnosis can be performed by enzymatic testing provided both parents have been studied, since pseudodeficiency resulting from polymorphisms is quite common. Genotyping of all patients is recommended, however, as prenatal diagnosis using molecular genetics is often advantageous. 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).

39.5.5 Treatment

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 [58]. In symptomatic infantile cases BMT gives very poor results, unless performed presymptomatically. Initial results with umbilical cord blood transplantation to 12- to 44-day-old babies were very promising [59]. However, long-term follow-up indicates that the transplant attenuates but does not cure the disease, and that over time the children develop slowly progressive motor and language deterioration along with somatic growth failure and persistent cognitive deficits [60, 61].

39.6 Metachromatic Leukodystrophy

39.6.1 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 [62, 63] is the most common. First symptoms appear between the ages of 1 and 2 years. Most children have begun to walk, although about 15% never walk independently. Around 14-16 months of age the children develop progressive difficulties in locomotion, weak lower limbs and falls. Examination usually shows hypotonia, and reduced or absent tendon reflexes due to peripheral neuropathy with extensor plantar responses. Walking and then standing soon become impossible. The child shows spastic quadriplegia, together with 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 [63] ranges between 3 and 14 years (some authors differentiate early 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 develop.

Two distinct types of **adult** MLD have been identified [64]. In the first group, patients have predominant motor disease, with pyramidal and cerebellar signs, dystonia and peripheral neuropathy, or isolated peripheral neuropathy [65]. In the second group, behavioural and psychiatric problems (often confused with schizophrenia) are the presenting symptoms, followed by dementia and spastic paresis [66].

39.6.2 Metabolic Derangement

The primary metabolic defect is a block in lysosomal degradation of sulfatide (or galactosylceramide-sulfate) and other sulfated glycolipids. In vivo, the sulfatide is presented to the enzyme arylsulfatase A (ASA) as a 1:1 complex with the SAP *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 possibly 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.

39.6.3 Genetics

More than 100 different mutations of the arylsulfatase A *ARSA* gene (22q13) are known [63]. The three more frequent alleles among European patients are 459+1G>A (severe phenotype), P426L (mild phenotype) and I179S (mild phenotype). There is a relatively good genotype-phenotype correlation [63]. Two very frequent polymorphisms of the *ARSA* gene, 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. They often occur jointly, but can also be found independently. In some countries, as many as 15% of the general population carry one such pseudodeficiency (*pd*) allele [63]. MLD due to *sap*-B deficiency is panethnic, but appears to be more frequent in Saudi Arabia [67]. These patients have mutations on the *PSAP* gene.

39.6.4 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 [68]. 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 [69, 70]. 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 arylsulfatase A in leukocytes (or cultured fibroblasts) using *p*-nitrocatechol-sulfate as a substrate constitutes the first biochemical test. Pseudodeficiency is a major pitfall [63]. Individuals homozygous for a *pd* allele (1–2% of the European population) have about 5-15% of normal ASA activity but no detectable clinical abnormality or pathology. The same applies to subjects compound heterozygotes for a disease-causing *mld* and a *pd* allele. Deficient ASA activity is therefore not enough to conclude to the diagnosis of MLD, even in a patient with a leukodystrophy. Molecular screening for the *pd* allele is useful, but the same allele may carry a *pd* polymorphism and an *mld* mutation. 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 prenatal diagnosis, and more generally in genetic counselling. In a newly diagnosed family, it has always been mandatory to study enzyme activity in both parents, but today full genotyping of the index case and study of parental DNA are also highly recommended. Prenatal testing of MLD by DNA analysis offers many advantages over enzyme activity measurement and is now the preferred strategy in many laboratories.

Another common cause of erroneous interpretation of an arylsulfatase A deficiency is the disease called **multiple sulfatase deficiency** (MSD), which is due to a primary deficiency in the formylglycine-generating enzyme (FGE) encoded by the *SUMF1* gene. It is therefore mandatory, whenever a deficiency of one sulfatase is found, to systematically measure the activity of another one (here, arylsulfatase B or iduronate-2-sulfatase etc.) to exclude a potential case of MSD, as the clinical picture can be misleading.

In MLD patients with *sap*-B deficiency, the regular in vitro ASA assay will not show a deficiency and studies of glycolipid excretion in urine are essential. The profile is pathognomonic, showing a combined MLD pattern and Fabry pattern. The ultimate diagnosis will require molecular analysis of the *PSAP* gene. Loading of labelled sulfatides in living fibroblasts is no longer used routinely.

39.6.5 Treatment and Prognosis

Symptomatic treatment of spasticity and of pain resulting from radiculopathy is important. Currently, there is no satisfactory treatment of any form of MLD. Allogeneic BMT has been performed in a number of cases. It is generally considered that adult-onset and juvenile-onset patients benefit from BMT, with slowing of the disease progression and improvement of cognitive functions, but challenging reports have appeared [63, 71-73]. Whether it is indicated in the late infantile form remains controversial [63]. Symptomatic patients are not candidates, but a number of still asymptomatic affected siblings have received BMT, with significant difference in survival and CNS involvement compared with untransplanted siblings. Unfortunately, BMT has no effect on the peripheral neuropathy. Experimental studies in the *ARSA* knockout mouse model suggest that achieving a high level of enzyme activity through ex vivo gene transfer might overcome this limitation [71]. Promising results of intracerebral AAV-ASA gene transfer in the mouse model are also leading the way to application of this in patients [74]. Intrathecal enzyme infusion also appears to be a potential therapeutic approach for the future.

39.7 Fabry Disease

39.7.1 Clinical Presentation

Fabry disease, the only X-linked sphingolipidosis, is associated with severe multiorgan dysfunction [75-77]. Its incidence has been estimated at 1 in 40,000 to 1 in 60,000 live births for males. Many heterozygous females are symptomatic. Although clinical onset occurs in childhood, disease presentation may be subtle, leading to retarded diagnosis or misdiagnosis. 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.

Episodic Fabry crises of acute pain may last hours to days. Unexplained bouts of fever and hypohidrosis, heat, cold and exercise intolerance, gastrointestinal problems and corneal dystrophy (cornea verticillata) that does not affect vision, are other manifestations. At this stage, renal function, urinary protein excretion and cardiac function and structure are generally still normal. 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. 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. Atypical variants with a milder, later onset phenotype have been described. The cardiac variant shows cardiac manifestations, with often mild proteinuria; a renal variant has been described in male hemizygotes, with kidney manifestations only (for a comprehensive review see [75]). Screenings have been conducted in high-risk populations [78].

39.7.2 Metabolic Derangement

The primary defect is a deficiency of the lysosomal enzyme α-galactosidase A, which releases galactose from ceramide trihexoside (globotriasylceramide, Gb3) and related glycosphingolipids (especially galabiosylceramide, Gb2), due to mutations of the *GLA* gene. 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, including cellular death, compromised energy metabolism, small vessel injury, KCa3.1 channel dysfunction in endothelial cells, oxidative stress, impaired autophagosome maturation, tissue ischaemia and development of irreversible cardiac and renal tissue fibrosis [75].

39.7.3 Genetics

Fabry disease has an X-linked recessive transmission. Close to 600 mutations of the *GLA* gene have been described. There are also numerous reported polymorphisms. Many mutations are private; a number are recurrent in specific countries. The mutation N215S seems to be associated with the cardiac variant. De novo mutations are rare.

39.7.4 Diagnostic Tests

In affected males with the classic or variant phenotype, the disease is readily diagnosed by showing an α-galactosidase A deficiency in leukocytes. Plasma or dried blood spots have also been advocated as better suited to large-scale screening, but subsequent confirmation in leukocytes is essential. In contrast, heterozygous females may have normal to low levels of activity, which means enzyme assay is not reliable for carrier detection. If the subject is related to a patient, molecular analysis is of course the test of choice. If not, full DNA sequencing is indicated, but incurs the risk of missed mutations or difficult interpretation due to the numerous polymorphisms. Studies of urinary glycolipids are useful. Large amounts of Gb3 and Gb2 are excreted by untreated male hemizygotes (except patients with a renal graft and those with a cardiac variant), and smaller but still significant amounts by 90% of heterozygote females, symptomatic or not. Measurement of urinary Gb3 can been used to monitor treatment [79, 80], and plasmatic lysoGb3 has been proposed as another interesting biomarker [81].

39.7.5 Treatment and Prognosis

The disease results in a significant reduction in life expectancy due to renal disease and cardiovascular or cerebrovascular complications [75-77]. There is also the psychosocial burden of a rare, chronic and progressive disease. It is essential that patients are diagnosed early, that the family receives genetic counselling and that proper treatment is given. 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. A disease-specific therapy with infusions of recombinant α-galactosidase A was introduced in 2001. Two essentially similar products, agalsidase alpha and agalsidase beta, exist. There is increasing evidence that ERT can halt disease progression. A detailed analysis of published reports and guidelines is reviewed in [75, 82]. Current research is aimed at developing an oral ther-

apy [20], and a phase III clinical trial using the chaperone migalastat hydrochloride is ongoing.

39.8 Farber Disease

39.8.1 Clinical Presentation

Farber lipogranulomatosis, a very rare disease, is clinically heterogeneous. Onset can be during infancy or much later, causing death within the 1st year or in some cases at an adult age. Fetal forms are known [83]. The most frequent signs are periarticular subcutaneous nodules and joint swelling, contractures and hoarseness due to laryngeal involvement. Hepatomegaly can be present, as can a macular cherry-red spot. Neurological manifestations are variable and may include severe psychomotor deterioration with seizures, or mild neurological involvement. Juvenile-onset patients may show neurological involvement only. The clinical description of later-onset cases is poorly documented.

39.8.2 Metabolic Derangement and Genetics

The deficiency of acid ceramidase leading to the storage of ceramide in various organs is due to mutations of the ceramidase gene *ASAH1* (8p21.3-22). Seventeen mutations are known.

39.8.3 Diagnostic Tests

Electron microscopy of excised nodule or a skin biopsy may reveal numerous 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 very few laboratories. Loading test in cultured fibroblasts using a precursor of ceramide (sphingomyelin of sulfatide) and subsequent study of ceramide turnover is also a specialised and elaborate test. Therefore, today, it is often easier and quicker to sequence the *ASAH1* gene directly.

39.8.4 Treatment and Prognosis

Currently there is no specific therapy. Symptomatic treatment is based on analgesics, corticotherapy and plastic surgery. Good results of BMT have been reported only in patients without central nervous system involvement [84, 85].

39.9 Prosaposin Deficiency

39.9.1 Clinical Presentation

The six 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 [86].

39.9.2 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. They act either by solubilising the substrate or by mediating enzyme binding to the membrane or modifying the enzyme conformation. The *PSAP* gene (10q21) encodes the prosaposin protein, which is transported to the lysosome where it is processed to four homologous proteins (*sap*-A to D). *sap*-A is a cofactor for degradation of galactosyl and lactosylceramide; its deficiency causes a Krabbe disease variant (1 case published); *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.

39.9.3 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 reveal a combined increase of glucosylceramide, lactosylceramide and ceramide. The loading test in living fibroblasts described for Farber disease will show a severe block in ceramide hydrolysis. In practice, the abnormal typical profile of

urinary glycolipids should lead directly to sequencing of the *PSAP* gene.

39.10 Niemann-Pick Disease Type C

Niemann-Pick type C disease (NP-C) is characterised by a complex defect in cellular lipid trafficking and therefore differs from the 'classic' sphingolipidoses.

39.10.1 Clinical Presentation

NP-C is panethnic, with an estimated incidence of 1 in 100,000 to 1 in 120,000 [87]. It includes former type D (a Nova Scotia *NPC1* isolate). The clinical course is extremely heterogeneous [87-89]. The age at presentation varies from the perinatal period to 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, but the systemic component may decrease with time, or be absent or minimal. Apart from a small subset of patients who die in the perinatal period and exceptional adult cases, all patients ultimately develop progressive and fatal neurological disease. Patients are often classified by age at onset of disease, but for periods other than perinatal some patients show only systemic signs, while others start to show neurological symptoms. For prognosis, a classification by neurological forms is more useful, since the age at onset of the neurological symptoms correlates with the following course and lifespan [87].

- **Perinatal Presentations**

Fetal hydrops or **fetal ascites** can occur. Liver involvement is often present in early life. About one third of NP-C patients have a **prolonged neonatal cholestatic icterus** with hepatosplenomegaly. In most patients, the icterus resolves spontaneously and only hepatosplenomegaly remains, but in a few 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 [87].

Neurological forms. In the severe early infantile neurological onset form, infants with a pre-existing hepatosplenomegaly (often with a history of neonatal icterus) 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 5 years [87].

Late-infantile- and juvenile-onset neurological forms (classic NP-C, 60-70% of cases) [87]. In the late infantile form, hepatosplenomegaly has generally been present for a varying period. The child often presents with gait problems and clumsiness at between 3 and 5 years of age, due to ataxia. Language delay is frequent. The motor problems worsen, and cognitive dysfunction appears. In the juvenile form, neurological symptoms appear between 6 and 15 years, and onset is more insidious and variable. Splenomegaly can be absent in 10-15% of cases. School problems, with difficulty in writing and impaired attention, are common and may lead to misdiagnosis. The child becomes clumsier and obviously has more learning disabilities, and ataxia become obvious. 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 with the classic form develop seizures, which may become difficult to treat. In a later stage, the patients develop pyramidal signs and spasticity and also swallowing problems. Most require gastrostomy. Death usually occurs between 7 and 12 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.

In **adult-onset** patients, presentation (as late as 60 years) is even more insidious. Some patients show severe ataxia, dystonia and dysarthria with variable cognitive dysfunction, while psychiatric symptoms and dementia are dominant in others [90]. Movement disorders are frequent, but epilepsy is rare in adult NP-C. These patients may not show splenomegaly or vertical gaze palsy. From recent data, it is evident that adult-onset patients have been (and probably still are) largely underdiagnosed.

39.10.2 Metabolic Derangement

When either the NPC1 or the NPC2 protein is nonfunctional, 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 [87, 88]. This specific abnormality constitutes the basis for biological diagnosis

of NP-C. Animal studies demonstrated nonredundant functional cooperativity of NPC1 and NPC2, supporting the view that the two proteins function in tandem or in sequence [87]. For handling of cholesterol, the sequential hands-off model proposed by the laboratory of Goldstein and Brown is very likely [91]. Nevertheless, the complete function(s) of NPC2, and above all of NPC1, remain unclear (for reviews see [87, 92]). The recent observation of a reduced Ca^{2+} release from acidic compartments in NPC fibroblasts suggests a potential primary role for another accumulated lipid, free sphingosine [93], but this remains hypothetical. Importantly, the pattern of accumulating lipids is different in brain and in non-neural organs. In extraneural organs sphingolipid accumulation is probably secondary to the cholesterol storage. On the other hand, neurons store only a minimal amount of cholesterol but significant amounts of glycolipids, including GM3 and GM2, and the question of which accumulation is primary in brain remains controversial [87, 92, 94]. Excess GM2 ganglioside in neurons is said to correlate with ectopic dendritogenesis and meganeurite formation [94, 95]. The mechanisms underlying other abnormalities, such as the formation of neurofibrillary tangles, neuroroaxonal dystrophy and, above all, the early and prominent loss of Purkinje cells are unknown [95].

39.10.3 Genetics

Approximately 95% of patients have mutations in the *NPC1* gene (18q11), which encodes a large transmembrane glycoprotein with late endosomal localisation. The remainder have mutations in the *NPC2* gene (14q24.3), which encodes a small soluble lysosomal protein that binds cholesterol with high affinity. More than 300 disease-causing mutations of the *NPC1* gene are known, as are >60 polymorphisms. I1061T is the most frequent allele in patients of western European descent, and P1007A the second most frequent one. About 40 families are known with mutations in the *NPC2* gene (20 described mutations) [87, 88].

39.10.4 Diagnostic Tests

Neuroimaging generally does not contribute to the diagnosis. Chitotriosidase in serum is often moderately elevated. The foamy histiocytes that may be found in bone marrow aspirates stain positive with filipin. The definitive biochemical diagnosis requires cultured fibroblasts. The pathognomonic accumulation of cholesterol in lysosomes is visualised by fluorescence microscopy after staining with filipin [87-89]. Filipin staining will give unequivocal results in about 80% of patients, but in the remainder, described as 'variants', the level of cholesterol is much lower, and the test requires conditioning of the cells in lipoprotein-deficient serum followed by a 24-h challenge with LDL-containing culture medium [96]. Even then, interpretation of results in variant cells can be very difficult [89] and may require gene sequencing. Because the filipin test can only be performed reliably in few laboratories, it is currently under discussion whether primary gene sequencing would not be preferable, considering the technological advances and diminishing cost. In fact, these two approaches should be regarded as complementary. Owing to the difficulty of finding certain mutations and the polymorphic nature of the genes, there will still be a significant number of cases in which the filipin study will be necessary for conclusive results. All patients should be genotyped, however, as only the molecular genetics approach is now used for prenatal diagnosis [87, 89]. Current research is aimed at discovering a screening test that can be carried out on a blood sample. At present, the measurement of plasma oxysterols constitutes the most promising lead [97, 98].

39.10.5 Treatment and Prognosis

Cataplectic attacks can be treated by clomipramine or CNS stimulants. Management of epilepsy, when present, is essential [89]. With progression, most patients will require tube feeding or gastrostomy. Cholesterol-lowering drugs have not shown any evidence of neurological benefit, but treatment of NPC1 mutant mice with miglustat led to a delay in onset of neurological symptoms and a prolonged life-span [20, 87]. Controlled clinical trials were therefore conducted in adolescents, adults and children, and long-term data have been reported [99, 100]. The disease course stabilised in 72% of patients treated for 1 year or more. Based on these results and those of an observational cohort study showing a slower rate of progression of the disease after treatment [101], miglustat has been approved for treatment of neurological manifestations in the EU since early 2009. Patients with late-onset forms generally emerged as the best responders. Indications, clinical utility and monitoring have been discussed [87, 89]. The treatment should preferably be started at an early stage of neurological manifestations [89]. Full evaluation of this drug will require longer term studies. There is no rationale for BMT in NPC1 patients [87], but the early outcome in one NPC2 patient is encouraging [102]. Among experimental therapies tested in the animal models (mouse and cat), the most promising results thus far

have been obtained with ß-hydroxypropyl-cyclodextrin [103]. Although this compound has received an orphan drug designation in the USA, transfer of its use to human patients is not straightforward [87]. Clinical trials in NP-C are hampered by clinical heterogeneity and lack of good objective markers of the disease.

39.11 Disorders of Sphingolipid Synthesis

Several disorders due to mutations of genes encoding proteins involved in sphingolipid synthesis have recently been described (▶ Chapter 35).

39.12 Neuronal Ceroid Lipofuscinoses

Neuronal ceroid lipofuscinoses (NCL) 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 wide heterogeneity of the disease. The different forms were grouped together because of their ultrastructural characteristics, i.e. the presence of autofluorescent lipopigments exhibiting a specific morphology. The past 15 years have seen major advances in the field of NCLs. The clinical diversity has been linked to a wide genetic heterogeneity, with eight different genes identified already and more to be found. Three of them encode enzymatic proteins, whilst the others encode transmembrane proteins whose function remains elusive. NCLs are now regarded as lysosomal storage diseases (LSDs) owing to the lysosomal accumulation of the lipopigments and, above all, the more recent localisation of several NCL proteins to the lysosome.

39.12.1 Clinical Presentation

NCL 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) [104]. 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 the 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 for each gene. Both classifications have been combined in the text below.

Infantile neuronal ceroid lipofuscinosis (INCL, Santavuori-Haltia) – the *CLN1* gene. 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 disturbance are seen in most children. Rapid visual impairment occurs as a result of optic atrophy and macular degeneration. Stereotyped hand movements may be present. Death takes place in the 1st decade of life. Even though mutations in the *CLN1* gene are mainly responsible for this classic infantile NCL, later onset forms (juvenile, adult) have also been described, which are probably due to less severe mutations [105].

Late infantile neuronal ceroid lipofuscinosis (LINCL, Jansky-Bielschowsky) – the *CLN2* gene. Children may be initially referred for delayed speech. Seizures, which may be of any type (partial, generalised tonic-clonic, absences) occur between 2 and 4 years of age. Ataxia, myoclonus and developmental regression become apparent, followed by a gradual decline in visual ability culminating in blindness by the age of 5 or 6 years. Death happens in middle childhood after a bedridden stage. Besides this classic LINCL, mutations in the *CLN2* gene have also been involved in atypical phenotypes with delayed onset and slower progression [105].

Classic juvenile neuronal ceroid lipofuscinosis (JNCL, Batten or Spielmeyer-Vogt) – the *CLN3* gene. 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 the ages of 5 and 18 years, and the predominant types are generalised 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 twenties. Other juvenile forms may be due to mutations on other genes.

Adult neuronal ceroid lipofuscinosis (ANCL, Kufs). Symptoms appear around age 30 years, with death occurring about 10 years later. Classically, two major forms have been delineated. Type A is characterised by myoclonic epilepsy, dementia and ataxia, while type B is marked by

behavioural changes and dementia, which may be associated with motor dysfunction. Retinal vision is not impaired in this form of NCL. Mutations in the *CLN1* gene have been found in some patients [105], but mutations in *CLN6* have recently been reported as the major cause of recessive type A adult NCL [106].

Variants of NCL. Among the numerous variants reported so far, some of them have been more specifically individualised.

Northern epilepsy or progressive epilepsy with mental retardation (EPMR) (*CLN8 gene*) is characterised by tonic-clonic seizures occurring between the ages of 5 and 10 years. Mental deterioration is observed 2-5 years after the onset of epilepsy. Vision problems are rare. Some patients are living to the age of well over 40 years [107].

The **Finnish variant** (*CLN5 gene*) usually begins around 4.5-6 years with clumsiness and difficulties in concentration. Visual impairment, ataxia and epilepsy appear a few years later. Life expectancy is between 13 and 35 years [105].

Other variants with earlier or later onset than the classic late infantile form have been described. The **Indo-European variant**, caused by mutations in the *CLN6 gene* [105] is seen in the Indian subcontinent, in South Europe and South America. A **Turkish variant** linked to the *CLN8* gene has been reported in a subset of late-infantile patients from consanguineous families [108]. More recently, the *CLN7/MFSD8* gene has been involved in other Turkish patients with LINCL [109]. Mutations in the *CLN7/MFSD8* gene are also present in LINCL patients from other countries.

The **congenital form** presents with microcephaly and seizures at birth, resulting in death within the first days of life. Mutations in the *CTSD* gene have been found in some patients [110].

39.12.2 Metabolic Derangement

Ceroid lipofuscinoses are characterised by the accumulation of autofluorescent ceroid lipopigments, mainly in neural tissues. The main components of this storage material are either saposins (SAP) A and D in infantile forms, or subunit c of mitochondrial ATP synthase (SCMAS) in late infantile and juvenile forms [111]. There are probably not disease-specific substrates, but secondary markers. Different hypotheses have been thought up to explain lipopigment accumulation: altered lysosomal function, disruption of autophagy and oxidative damages of proteins [111]. Cell biology and model organisms have been instrumental in the approach to the functional biology of NCL proteins [112]. These proteins are mainly localised in the lysosome, but CLN6 and CLN8 are found in the endoplasmic reticulum [113]. Four of them are soluble (CLN1, CLN2, CLN5, CTSD); the others are transmembrane proteins (CLN3, CLN6, CLN7, CLN8). The function of soluble proteins is beginning to be clarified, whereas that of transmembrane proteins is still poorly understood [113, 114]. Interactions have been described between the different NCL proteins, but whether or not these are involved in a common pathway is uncertain [114]. Moreover, the relation between NCL protein dysfunction, accumulated material and neuronal alterations remains to be determined.

39.12.3 Genetics

NCL are usually inherited in an autosomal recessive manner (except some adult forms that are reported to be dominantly transmitted). They result from mutations in different genes encoding the various NCL proteins [105, 113]. To date, eight genes have been delineated, but other genes are still to be discovered. The *CLN1* gene, located on 1p32, encodes the enzyme palmitoyl protein thioesterase 1 (PPT1). Numerous mutations have been reported on this gene, but p.Arg122W and p.Arg151X are common in Finnish and non-Finnish patients, respectively. The *CLN2* gene (11p15) encodes the enzyme tripeptidyl peptidase I (TPP1). Two mutations are common on this gene: c.509-1G>C and p.Arg208X, but more than 70 private mutations have also been described. A 1-kb deletion (c.461-280_677+382del966) is particularly frequent (80-90% of alleles) on the *CLN3* gene, located on 16p12. Concerning the *CLN5* gene (13q22), the mutation p.Tyr392X is frequent in the Finnish population, but different mutations have been found in other countries. Northern epilepsy is mainly due to the common mutation p.Arg24Gly in the *CLN8* gene (8p23). Other abnormalities have been described on the same gene in patients presenting with late-infantile variants. Numerous mutations have been reported on the *CLN6* (15q21-23), the *CLN7/MFSD8* (4q28.1-28.2) and the *CLN10*/*CTSD* (11p15.5) genes. The spectrum of NCL mutations are given in the NCL Mutation Database (http://www.ucl.ac.uk/ncl/).

39.12.4 Diagnostic Tests

Electrophysiological studies are helpful to establish the diagnosis of NCL. The electroretinogram (ERG) is generally diminished at presentation, and it becomes rapidly extinguished. In INCL, the first abnormality in the

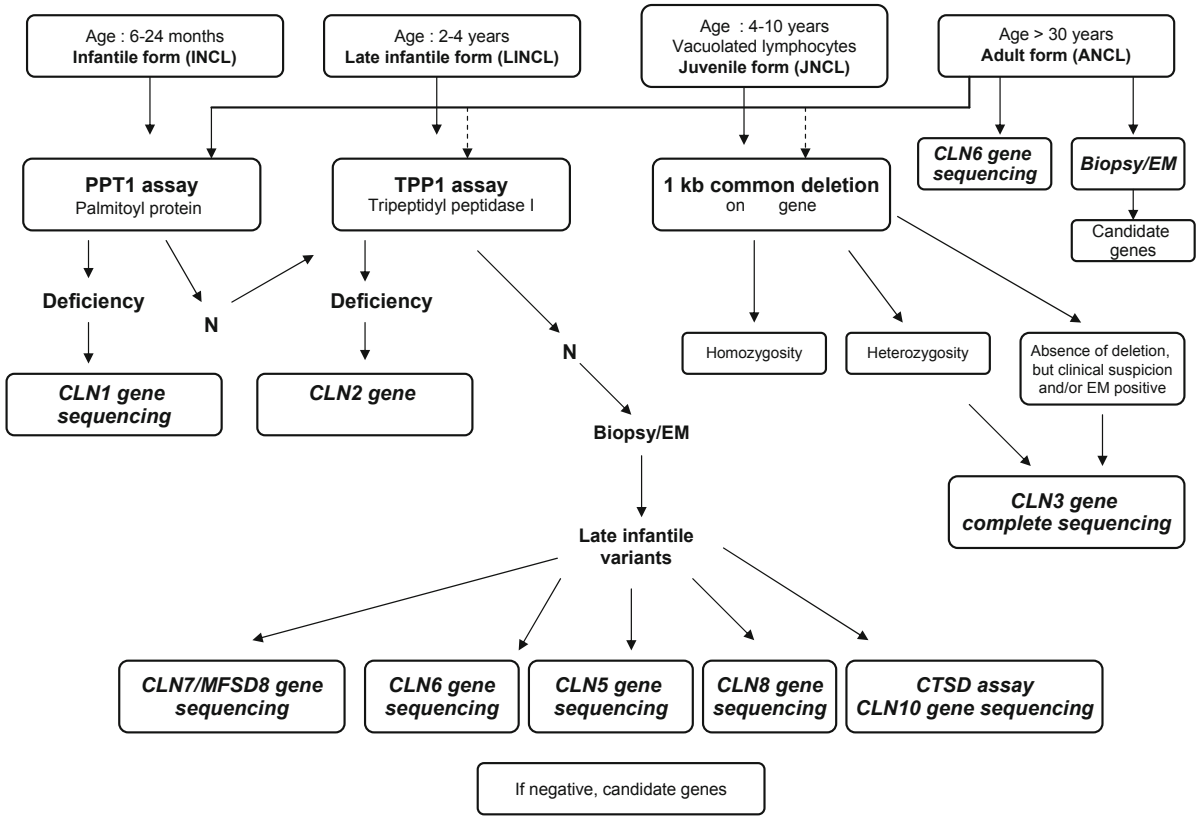
electroencephalogram (EEG) is the disappearance of the eye-opening/-closing reaction, followed by a loss of sleep spindles. Then, the EEG rapidly becomes flat. In LINCL, an occipital photosensitive response to photic stimulation at 1-2 Hz with the eyes open is present. Magnetic resonance imaging (MRI) shows progressive brain atrophy, which is particularly severe in INCL, sometimes beginning in the cerebellum in other forms.

Vacuolated lymphocytes are only present in the juvenile form (*CLN3* gene). 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 the *CLN1* or the *CLN10/CTSD* gene. Curvilinear (CV) profiles are present in the classic LINCL (*CLN2*) and fingerprint (FP) profiles 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 and to direct the genetic studies.

For the *CLN1* and *CLN2* loci, the diagnosis is established by measuring respectively the palmitoyl protein thioesterase and the tripeptidyl peptidase I activities, either on leukocytes or on cultured fibroblasts, using specific artificial fluorogenic substrates. The disease-causing mutations are then characterised by complete sequencing of the corresponding genes. The diagnosis strategy is similar for the *CLN10/CTSD* gene encoding cathepsin D (rare cases). For the other genes, complete sequencing is performed directly, except for the *CLN3* gene where it is preceded by the rapid detection (PCR) of the 1 kb common deletion [115]. A diagnostic algorithm is shown in ◘ Fig. 39.2. Prenatal diagnosis is possible using specific enzymatic assay and/or detection of the mutation(s) previously determined in the index case.

39.12.5 Treatment and Prognosis

Antiepileptic drugs need to be selected with caution. Lamotrigine is usually efficient against seizures. Levetiracetam may also be beneficial, but carbamazepine and phenytoin can worsen the symptoms. Diazepines should be useful for seizures, anxiety and sleep disturbances. Gastrostomy



◘ **Fig. 39.2.** Algorithm for diagnosis of the neuronal ceroid lipofuscinoses

is used to maintain the nutritional status in the late stages of the disease. No specific treatment is available, but ERT and gene transfer approaches are under investigation [116]. Clinical trials based on direct CNS administration of replication-deficient adeno-associated vectors (AAV) are ongoing (http://clinicaltrials.gov/) [117].

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Mucopolysaccharidoses and Oligosaccharidoses

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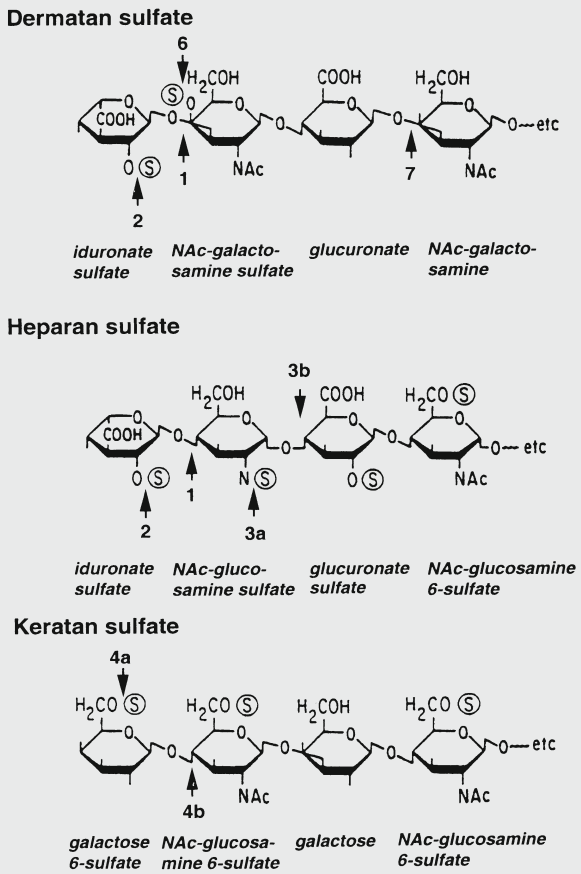
Mucopolysaccharides

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. 40.1). 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. 40.1.** Main repeating units in mucopolysaccharides 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*-acetylglucosaminidase (MPS IIIb: Sanfilippo B disease); **4a**, *N*-acetylgalactosamine-6-sulfatase (MPS IVa: Morquio A disease); **4b**, β-galactosidase (MPS IVb: Morquio B disease); **6**, NAc-galactosamine 4-sulfatase (MPS VI: Maroteaux-Lamy disease); **7**, β-glucuronidase (MPS VII: Sly disease)

Genetic defects in enzymes that are involved in the lysosomal degradation of the mucopolysaccharides (glycosaminoglycans, GAGs) (◘ Fig. 40.1) and the oligosaccharide chains of glycoproteins (◘ Fig. 40.8) lead to chronic and progressive storage disorders that share many clinical features. These vary from facial dysmorphism, bone dysplasia (dysostosis multiplex), hepatosplenomegaly, neurological abnormalities, developmental regression and a reduced life expectancy at the severe end of the clinical spectrum, to an almost normal clinical phenotype and life span in patients with more attenuated disease. 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 partially degraded GAG or oligosaccharide in urine and confirmed by specific enzyme assays in serum, leukocytes or skin fibroblasts.

For the majority of disorders treatment is palliative, but there have been important advances in the use of specific enzyme replacement therapy strategies for some MPS and this is an area of very rapid development. In addition, haematopoietic stem cell transplantation (HSCT) can improve outcome in carefully selected patients with MPS (especially MPS IH, *Hurler syndrome*), but this procedure is associated with significant morbidity and mortality.

Gene augmentation/transfer using a variety of vectors has been successful in cultured cells and animal models but has not yet been successfully performed in a human patient with one of these disorders.

It is important to remember that prenatal diagnosis is possible for all the MPS and oligosaccharidoses.

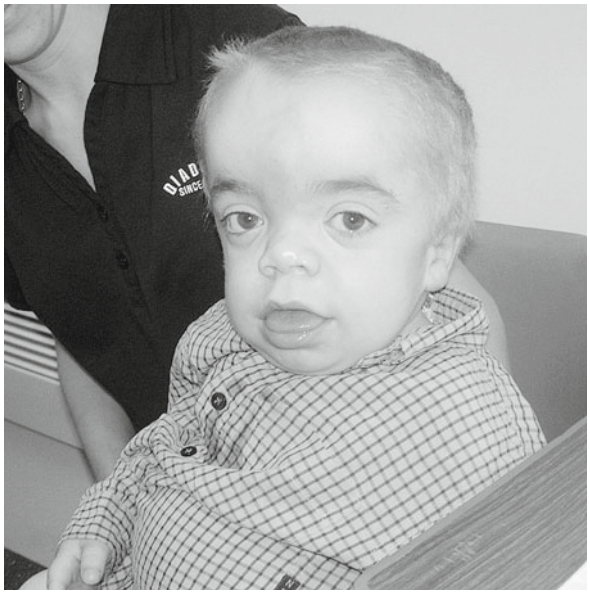


Fig. 40.2. Facial features of Hurler syndrome (MPS IH)

40.1 Clinical Presentation

40.1.1 Mucopolysaccharidoses

Mucopolysaccharidoses (MPS), like all lysosomal storage diseases, are chronic, progressive multisystem disorders. Affected infants are usually normal at birth, and the disease is only diagnosed as the phenotype evolves with time. Infants with an MPS-like phenotype present at birth are most likely to have mucolipidosis type II (I-cell disease). There is very wide clinical heterogeneity within this group of disorders.

In general the MPS disorders present in one of three ways:

1. As a dysmorphic syndrome e.g. MPS IH (*Hurler*), MPS II (*Hunter*), MPS VI (*Maroteaux-Lamy*);
2. With learning difficulties, behavioral disturbance and dementia e.g. MPS III (*Sanfilippo*);
3. As a severe bone dysplasia, e.g. MPS IV (*Morquio*).

Patients with MPS often have a facial appearance that is characteristically labelled coarse, although most parents find the term objectionable. A combination of subcutaneous storage and involvement of the facial bones in the dysostosis produces the typical appearance, seen in its most developed form in MPS IH (*Hurler syndrome*, Fig. 40.2). Underdevelopment of the mid-facial skeleton and the firm puffiness associated with subcutaneous storage results in a flat nasal bridge and blurring of the facial features. The lips and tongue are thickened, and the hair is often abundant and dull. The persistent nasal discharge detracts further from the child's general appearance. A dark synophyris is a characteristic finding and affected children are often hirsute. The facial phenotype is much less obvious in patients with MPS III and absent in patients with MPS IV. A brief clinical summary for all of the disorders discussed is given in Table 40.1.

- **Hurler Syndrome (MPS IH) and Scheie Disease (MPS IS)**

Patients with MPS I have deficiency of the enzyme α-l-iduronidase (Fig. 40.1) and accumulate the glycosaminoglycans (GAGs) dermatan and heparan sulfate (DS, HS). Infants with severe disease (MPS 1H, *Hurler syndrome*) are usually diagnosed in the 1st year of life [1]. Upper airway obstruction and frequent ear, nose and throat infections dominate the clinical picture at an early stage, and then the full clinical picture of short stature, hepatosplenomegaly, increasing facial dysmorphism, cardiac disease, progressive learning difficulties and corneal clouding evolves over the 2nd and 3rd years of life. The bone dysplasia that occurs in MPS and other lysosomal storage disorders, dysostosis multiplex, is most florid in MPS IH and includes abnormalities in the skull (enlarged sella turcica, scaphiocephaly; Fig. 40.3), broad ribs, hook-shaped vertebrae (Fig. 40.4), prominent pointing of the metacarpals (Fig. 40.5) and underdevelopment of the pelvic bones. Patients with severe MPS I usually die before the age of 10 years as a result of cardiorespiratory disease.

At the other end of this clinical spectrum patients with *Scheie disease* (MPS IS) are intellectually normal,

Table 40.1. Mucopolysaccharidoses (MPS), mucolipidoses (ML) and oligosaccharidoses (OS) – diagnostic data

Disease	Enzyme deficiency	Storage material	Chromosome location	Gene mutations	Screening test	Diagnostic test	Prenatal diagnosis	Main clinical features
Mucopolysaccharidoses								
MPS I (Hurler, Scheie, Hurler/Scheie)	Iduronidase	DS, HS	4p16.3	W402X, Q70X plus many others	Urine GAGs	WBC enzyme assay	CVB[1]	HSM, CNS, SD, DYS, OPH, CAR
MPS II (Hunter)	Iduronate-2-sulfatase	DS, HS	Xq27-28	No common mutations	Urine GAGs	Plasma enzyme assay	CVB[2]	HSM, CNS, SD, DYS, OPH, CAR, SK
MPS III (Sanfilippo)								
IIIA	Heparan-*N*-sulfatase	HS	17q25.3	R245H, R74C and many others	Urine GAGs	WBC enzyme assay	CVB	CNS, SD (+/-), DYS (+/-)
IIIB	*N*-Acetylglucosaminidase	HS	17q21.1	No common mutations	Urine GAGs	Plasma enzyme assay	CVB	CNS, SD (+/-), DYS (+/-)
IIIC	Acetyl CoA glucosamine *N*-acetyl transferase	HS	8p11	Unknown	Urine GAGs	WBC enzyme assay	CVB	CNS, SD (+/-), DYS (+/-)
IIID	*N*-Acetyl-glucosamine-6-sulfatase	HS	12q14	Very few patients studied	Urine GAGs	WBC enzyme assay	CVB	CNS, SD (+/-), DYS (+/-)
MPS IV (Morquio)								
IVA	*N*-Acetylgalactosamine-6-sulfatase	KS	16q24	I113F (UK and Ireland)	Urine GAGs	WBC enzyme assay	CVB	SD, CAR, OPH (+/-)
IVB	β-Galactosidase	KS	3p21-pter	No common mutations	Urine GAGs	WBC enzyme assay	CVB	SD, CAR
MPS VI (Maroteaux-Lamy)	N-Acetylgalactosamine-4-sulfatase	DS	5q12	No common mutations	Urine GAGs	WBC enzyme assay	CVB[3]	HSM, SD, DYS, OPH, CAR
MPS VII (Sly)	β-Glucuronidase	HS, DS	7q21	Very few patients studied	Urine GAGs	WBC enzyme assay	CVB	HF, HSM, CNS, SD, DYS, OPH, CAR
MPS IX	Hyaluronidase	HA	3p21.3	Very few patients studied	None	Cultured cells	Unknown	OA
Mucolipidoses								
ML I (sialidosis I)	Neuraminidase	Sialic acid	6p21.3	No common mutations	Urine sialic acid	Cultured cells	Cultured cells	CNS, CRS, SD (+/-)
ML II (I cell)	Transferase[4]	Many	12q23.3	No common mutations	Urine oligos	Plasma enzyme assay	Cultured cells or AF	HSM, CNS, SD, DYS, OPH, CAR

ML III (pseudo-Hurler)	Transferase[4]							
IIIA	As ML II	Many	12q23.3	Very few patients studied	Urine oligos	Plasma enzyme assay	Cultured cells or AF	HSM (+/-), CNS (+/-), SD, DYS (+/-), CAR
IIIC	Transferase – δ-subunit	Many	16p13.3	Very few patients studied	Urine oligos	Plasma enzyme assay	Cultured cells or AF	as ML III A
ML IV	TRP	Unknown	19p13.2-13.3	R750W (20%)	None	DNA	DNA	CNS, OPH
Oligosaccharidoses								
α-Mannosidosis	α-Mannosidase	α-Mannosides	19q12	R750W (20%)	Urine oligos	WBC enzyme assay	CVB	HSM, SD, DYS, CAR, CNS (+/-)
β-Mannosidosis	β-Mannosidase	β-Mannosides	4q22	Very few patients studied	Urine oligos	WBC enzyme assay	CVB	CNS, HSM (+/-)
Fucosidosis	Fucosidase	Fucosides glycolipids	1p24	No common mutations	Urine oligos	WBC enzyme assay	CVB	CNS, SKA
Aspartylglucosaminuria	Aspartylglucosaminidase	Aspartyl-glucosamine	4q32	C163S (90% of Finnish patients)	Urine oligos	WBC enzyme assay	CVB	CNS, DYS, SD (+/-)
Schindler disease	α-*N*-Acetylgalactosaminidase	*N*-Acetylgalactosamineglycolipids	22q13	Very few patients studied	Urine oligos	WBC enzyme assay	CVB	CNS, SD, SKA
ISSD (infantile sialic acid storage disease)	Sialic acid transporter	Sialic acid	6q14-q15	R39C/other mutation	Urine oligos (some patients test negative)	Cultured cells	AF	HF, DYS, HSM, CNS, SD
Salla disease	As ISSD	As ISSSD	As ISSD	R39C homozygotes	As ISSD	As ISSD	As ISSD	CNS (+/-)
Cerebellar ataxia with elevated CSF sialic acid (CAFSA)	Unknown	Sialic acid	Unknown	Unknown	CSF sialic acid	Unknown	Unknown	CA, CNS, PN
Galactosialidosis	Neuraminidase and β-galactosidase protective protein	Oligosaccharides, sialic acid	20q13.3	Very few patients studied	Urine oligos	Cultured cells	Cultured cells	HF, CNS, SD, HSM

Storage material: *DS*, dermatan sulfate; *HA*, hyaluronic acid; *HS*, heparan sulfate; *KS*, keratan sulfate. Screening test: *GAGs*, glycosaminoglycans; *oligos*, oligosaccharides. Diagnostic test: *WBC*, white blood cells. Prenatal diagnosis: *AF*, amniotic fluid; *CVB*, chorionic villus biopsy. Main clinical features: *CA, cerebellar ataxia; CAR*, cardiac disease; *CNS*, central nervous system regression; *CRS*, cherry red spot; *DNA*, mutation analysis; *DYS*, dysmorphic appearance; *HF*, hydrops fetalis; *HSM*, hepatosplenomegaly; *OPH*, eye signs: corneal clouding or ophthalmoplegia; *OA*, osteoarthritis; *PN, peripheral neuropathy; SD*, dysostosis multiplex; *SK*, dermatological signs; *SKA*, angiokeratoma; *TRP*, transient receptor potential. (+/-), sign not always present or mild. [1] Low activity in CVB – caution re: contamination with maternal decidua. [2] Always do fetal sexing, as some unaffected female fetuses will have very low enzyme results. [3] Difficult because of cross-reactivity from other sulfatases. [4] Lysosomal UDP-*N*-acetylglucosamine-*l*-phosphotransferase. ML II and IIIA have defects in α or β subunits, ML III C has defects in γ subunit (Grabowski 2008)

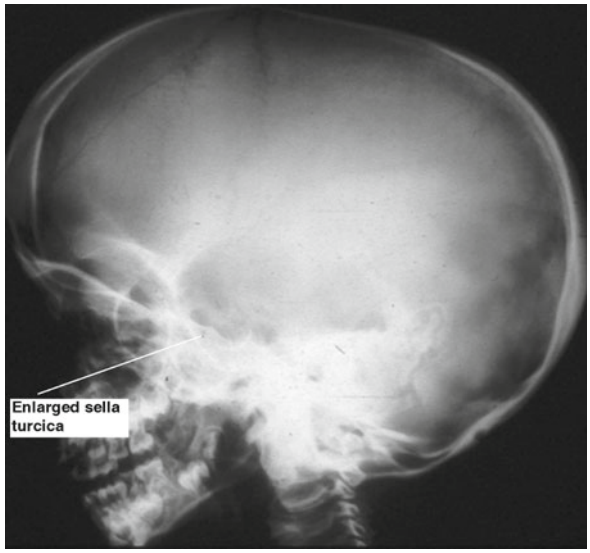


Fig. 40.3. Dysostosis multiplex in Hurler syndrome (MPS 1H): scaphiocephalic skull with expanded sella turcica

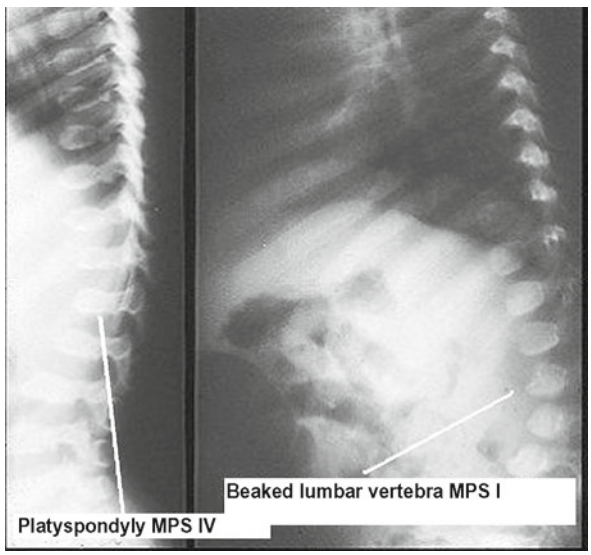


Fig. 40.4. Dysostosis multiplex: platospondyly in Morquio disease (MPS IV) and beaked lumbar vertebra in Hurler syndrome (MPS IH)

often reach normal height and can live a normal life span, although many patients become disabled as a result of degenerative joint disease, corneal opacity and cardiac valve lesions.

The symptoms of patients between these two extremes can be extremely variable (*Hurler-Scheie*, MPS I H-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 little or no intellectual dysfunction. Untreated, the condition usually leads to death from cardiac disease during the 2nd or 3rd decade of life.

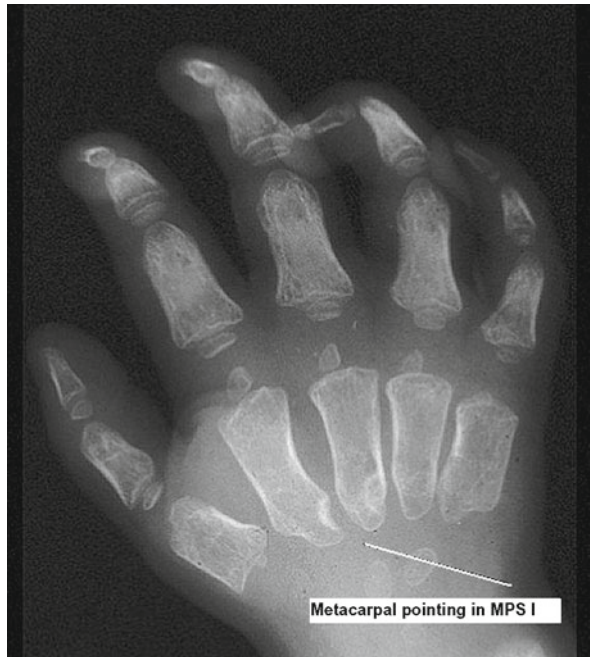


Fig. 40.5. Dysostosis multiplex in Hurler syndrome (MPS IH): proximal pointing of the metacarpals

Hunter Syndrome (MPS II)

MPS II (iduronate-2-sulfatase deficiency, *Hunter syndrome*) differs from other MPS in that its inheritance is X-linked recessive. Unlike Fabry disease, 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. Prominent Mongolian blue spots and a characteristic papular rash are other features that are prominent in severe MPS II (Fig. 40.6). 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. Attenuated patients with MPS I, II and VI are at risk of developing cervical myelopathy due to dural thickening (pachymeningitis cervicalis) and thickening of the transverse ligaments even if the craniocervical junction has a stable arrangement (Fig. 40.7). This often presents insidiously with loss of endurance before more obvious signs of ascending paralysis become apparent.

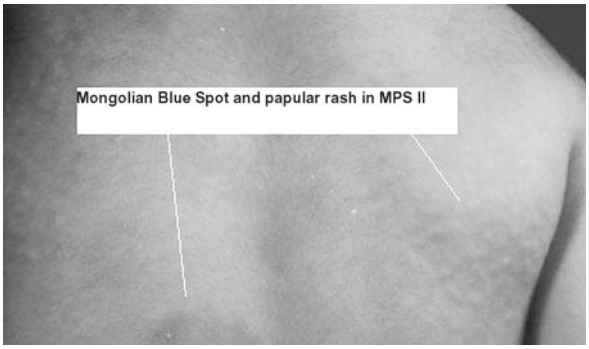


Fig. 40.6. Papular rash and Mongolian blue spot in Hunter syndrome (MPS II)

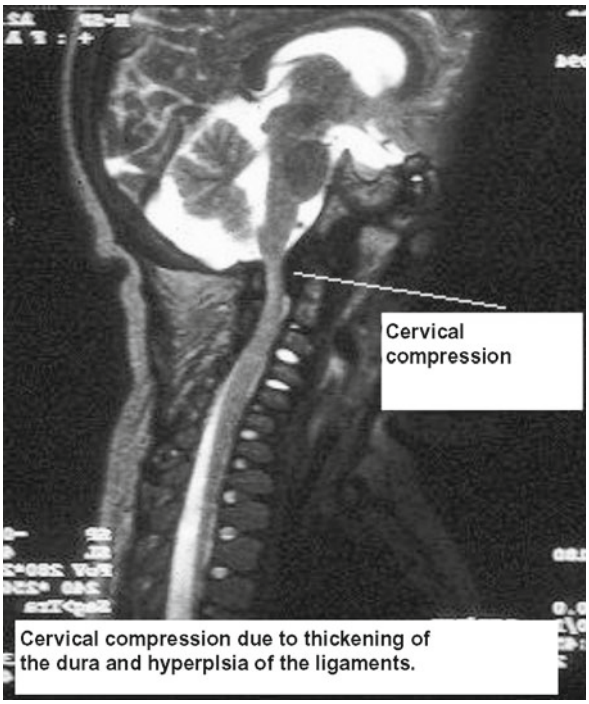


Fig. 40.7. MRI scan of the craniocervical junction demonstrating cervical cord compression in Hunter syndrome (MPS II)

Sanfilippo Syndrome (MPS III)

Patients with all four subtypes of MPS III (A, B, C and D, *Sanfilippo syndrome*) have a defect in the catabolism of heparan sulfate. This results in a disorder which primarily affects the central nervous system, whereas somatic abnormalities are usually mild. This often leads to a considerable delay in diagnosis. The condition has three phases [2]. 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, characterised by hyperactivity, challenging behaviour, and profound sleep disturbance. The third phase of the illness (usually after the first decade) is associated with continuing loss of skills and slow deterioration into a vegetative state, death usually occurring early in the 3rd decade. As in all the other MPS there is considerable heterogeneity, and not all patients will follow this pattern of deterioration. Patients with MPS III may be of normal height, have mild dysostosis multiplex and often develop seizures in the second or third phase of their illness.

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 (Fig. 40.4), hip dysplasia and genu valgum. Intellectual impairment does not occur in MPS IV, but the height prognosis is very poor, with affected adults ranging from 95 to 105 cm when fully grown. Odontoid dysplasia is often associated with atlanto-axial subluxation and renders the patients vulnerable to acute or chronic cervical cord compression. In *Morquio B* (MPS IVB, β-galactosidase deficiency, GM_1-gangliosidosis) the skeletal involvement is not as pronounced, but the patients have prominent central nervous system disease and a slowly progressive neurodegenerative course.

Maroteaux-Lamy Syndrome (MPS VI)

Patients with MPS VI (*N*-acetylgalactosamine-4-sulfatase deficiency, *Maroteaux-Lamy syndrome*) have somatic features resembling MPS I, but without neurological impairment. This disorder is rare, but it is important to recognise it early, as enzyme replacement therapy (ERT) is available to treat affected patients.

Sly Syndrome (MPS VII)

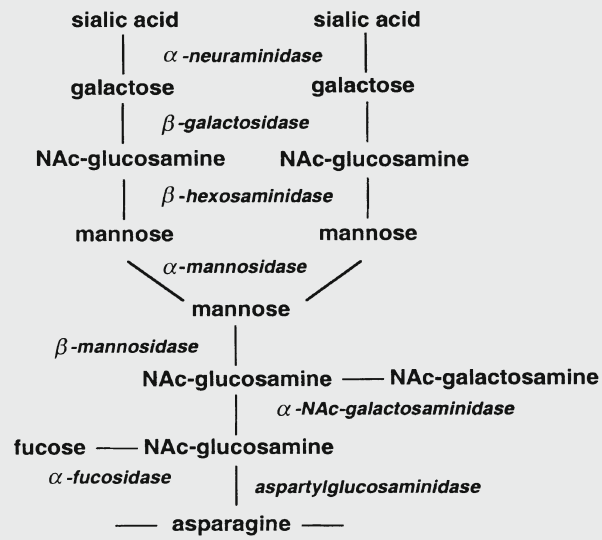
MPS VII (β-glucuronidase deficiency, *Sly syndrome*) is a very variable disorder, which has hydrops fetalis as its most common presentation. Patients who survive pregnancy have a clinical disease similar to MPS I, including the same degree of clinical heterogeneity.

Natowicz Syndrome (MPS IX)

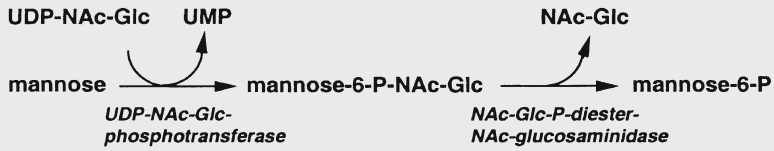
The newest MPS disorder, MPS IX (hyaluronidase deficiency, *Natowicz syndrome*), has been fully characterised in two families. In one patient the disorder presented with short stature and periarticular soft masses [3,4], whereas in the other the condition mimicked familial juvenial idiopatic arthritis [5].

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. 40.8). 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. 40.9). 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 enymes. Defects of the synthesis of the oligosaccharide chains are discussed in ► Chapter 42.



◘ **Fig. 40.8.** General composite example of a glycoprotein oligosaccharide chain. *NAc*, *N*-acetyl. Degradative enzymes are listed in *italics*



◘ **Fig. 40.9.** Synthesis of the mannose-6-phosphate recognition marker. *NAc-Glc*, *N*-acetylglucosamine; *UDP*, uridine diphosphate; *UMP*, uridine monophosphate. Enzymes are listed in *italics*

40.1.2 Oligosaccharidoses

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.

- **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 [6]. *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 [7].

- **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 severe neurodegenerative disorder, often with seizures and a mild dysostosis. Affected patients often exhibit prominent, widespread angiokeratomas, and as in all oligosaccharidoses the clinical course of the patients can be variable [8].

- **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 proteolytic processing. The clinical features of affected patients includes hydrops fetalis as well as a more slowly progressive disorder associated with learning difficulties, dysostosis multiplex and corneal opacity.

- **Transport Defects**

The allelic disorders *Salla disease* and *infantile free sialic acid storage disease (ISSD)* result from mutations in the *SLC17A5* gene 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 survive into middle age [9]. Mutations in the *SLC17A5* gene have also been reported in two siblings with mental retardation and hypomyelination [10]. In these patients free sialic acid was increased in CSF, but in contrast urinary excretion of free sialic acid was normal when tested on several occasions by different methods. 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 [11]. The molecular basis of this new disease is yet to be established.

- **Aspartylglucosaminuria**

This disorder is due to a deficiency of aspartylglucosaminidase and has a high prevalence in Finland but is rare in other countries. A characteristic facial dysmorphism has been described (hypertelorism, a short and broad nose, simple ears with small or missing lobule, and thick lips [12]), and there is slowly progressive psychomotor retardation, with death in middle age [13].

- **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. There are discrepancies between genotype and phenotype in the disease, and it has been suggested that other factors (apart from the enzyme deficiency) may contribute to the severe neurological findings in early-onset patients [14].

- **Sialidosis (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 neuraminidase. 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 the comparatively slowly progressive cherry-red-spot myoclonus syndrome.

- **I-Cell Disease (ML II) and Pseudo-Hurler (ML III)**

Patients with ML II have a Hurler-like phenotype often presenting in the newborn period. 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 and are often the most important indicators of prognosis. Most patients die in the 1st decade of life. 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.

- **Pycnodysostosis**

Cathepsin K is a recently identified lysosomal cysteine proteinase abundant in osteoclasts, where it is felt to play 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 [15]. 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 are also common.

40.2 Metabolic Derangements

A summary of the metabolic derangements (enzyme deficiency and storage material) in the MPS, oligosaccharidoses and mucolipidoses is given in ◘ Table 40.1.

The vast majority of disorders are defects of single enzymes involved in the degradation of mucopolysaccharides (◘ Fig. 40.1) or oligosaccharides (◘ Fig. 40.8). 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. 40.9), the enzyme necessary for synthesis of the recognition marker required to target various newly formed lysosomal enzymes to the lysosomes. As a result, the enzymes are secreted into the extracellular space, where high activity is found; inside the cells the enzyme levels are considerably reduced. In the transport defects the gene encoding the lysosomal membrane protein sialin is defective. Urinary excretion of 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 [16].

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 [17].

40.3 Genetics

With the exception of MPS II (*Hunter syndrome*), the disorders in this group are inherited in an autosomal recessive manner. ◘ Table 40.1 gives details of the genes involved and also of common mutations where present. 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.

40.4 Diagnostic Tests

The diagnosis of most MPS and oligosaccharidoses is based on clinical suspicion, supported by appropriate clinical and radiological examinations followed by urinary examination for glycosaminoglycan (GAG) and oligosaccharide excretion and then specific enzyme assay, usually on white blood cells. The diagnostic tests, including screening and both prenatal and postnatal diagnosis, are shown in ◘ Table 40.1. Many different methods have been used in the initial biochemical screening of these patients. As most patients (except those with MPS IX) excrete increased amounts of GAG or oligosaccharides, analysis of urine for the presence of these substances is the most common first step in the diagnostic process. Spot tests are inexpensive and can be performed rapidly, but unfortunately they can be unreliable and may miss patients with MPS III and MPS IV. For quantitative measurement of urine GAG, several tests are available (e.g. determination of uronic acid based on the carbazol method or the spectrophotometric assay using the dye dimethylmethylene blue). Two-dimensional electrophoresis of GAG extracted from urine allows the clearest discrimination between different GAG species. 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. Patients with ML II and III are often missed, and therefore the definitive diagnosis should be confirmed by the appropriate enzyme assays (◘ Table 40.1).

Increasing development of high-throughput multiplex enzyme assays is making the possibility of newborn screening a reality for LSDs, including the MPS. Although this approach has yet to win universal approval it would certainly seem to offer the best prospects for successful early therapy [18].

40.5 Treatment and Prognosis

Palliative care remains an important aspect of the holistic management of patients with these disorders. For some conditions it is the only available therapy. Multidisciplinary management is essential, and patients are best managed in specialist centres with access to a comprehensive range of clinical and supporting services. The careful use of medication to treat sleep disturbance (e.g. melatonin) and challenging behaviour (e.g. risperidone) is important in those conditions primarily affecting the brain (e.g. MPS III). 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. A recent report has suggested that personalised growth hormone (GH) therapy (dosage based on IGF-1 levels) leads to near-normal stature and skeletal proportions in patients with pycnodysostosis. The mechanism by which GH is defective in this condition remains speculative [19].

Attempts at curative treatment for the group of disorders discussed in this chapter currently include haematopoietic stem cell transplantation (HSCT) and enzyme replacement therapy (ERT). Substrate reduction therapy (SRT) has been attempted in murine models using a variety of products, the most successful being Genistein (4',5,7-trihydroxyisoflavone or 5,7-dihydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one), the most abundant isoflavone in soy. One of its many actions is to inhibit the synthesis of GAGs demonstrated in skin fibroblasts from patients with mucopolysaccharidoses and in mouse models of MPS IIIB [20].

Gene augmentation (gene therapy) has not been successfully applied in affected humans, although results in some animal studies are promising.

The largest experience of the use of HSCT is in MPS I (severe variant, *Hurler syndrome*). A number of cell sources have been used, including umbilical cord blood, and this whole area has been recently reviewed [21]. Results are variable, and although developmental progress can be preserved the patients often have severe residual orthopaedic problems [22]. In addition, the success of this therapy is limited by significant morbidity and mortality, although results are improving. Experience in other MPS disorders is more limited, and efficacy has not been clearly demonstrated in any of the other MPS conditions, with the exception of MPS VI.

ERT is a safer approach to treatment for these disorders, but is limited by an inability of the enzyme to cross the blood-brain barrier when given intravenously. Long-term extension study data is now available for Aldurazyme (MPS I, Biomarin/Genzyme) [23], and increasingly ERT is being used in combination with HSCT to try and improve outcome [24]. Elaprase (Shire) is also available to treat the non-neurological manifestations of MPS II [25], and Naglazyme (Biomarin) is licensed for the treatment of MPS VI [26]. ERT is in development for both MPS IV and α-mannosidosis. Limitations of this approach to therapy include the development of neutralising or blocking antibodies and the very high cost of therapy [27]. In an attempt to treat the neurological manifestations of MPS by ERT intrathecal ERT has been developed [28], and some of the approaches proposed for neuronopathic LSDs may also be of relevance [29].

Successful gene augmentation trials have not yet been demonstrated in patients with this group of disorders. Problems with sustained gene expression have hindered progress, but this method of treatment may be the most likely to be successful in disorders with prominent central nervous system components. Animal models are proving a valuable resource to evaluate early studies of gene augmentation [30], and it is only a matter of time before this is revisited in the human.

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Peroxisomal Disorders

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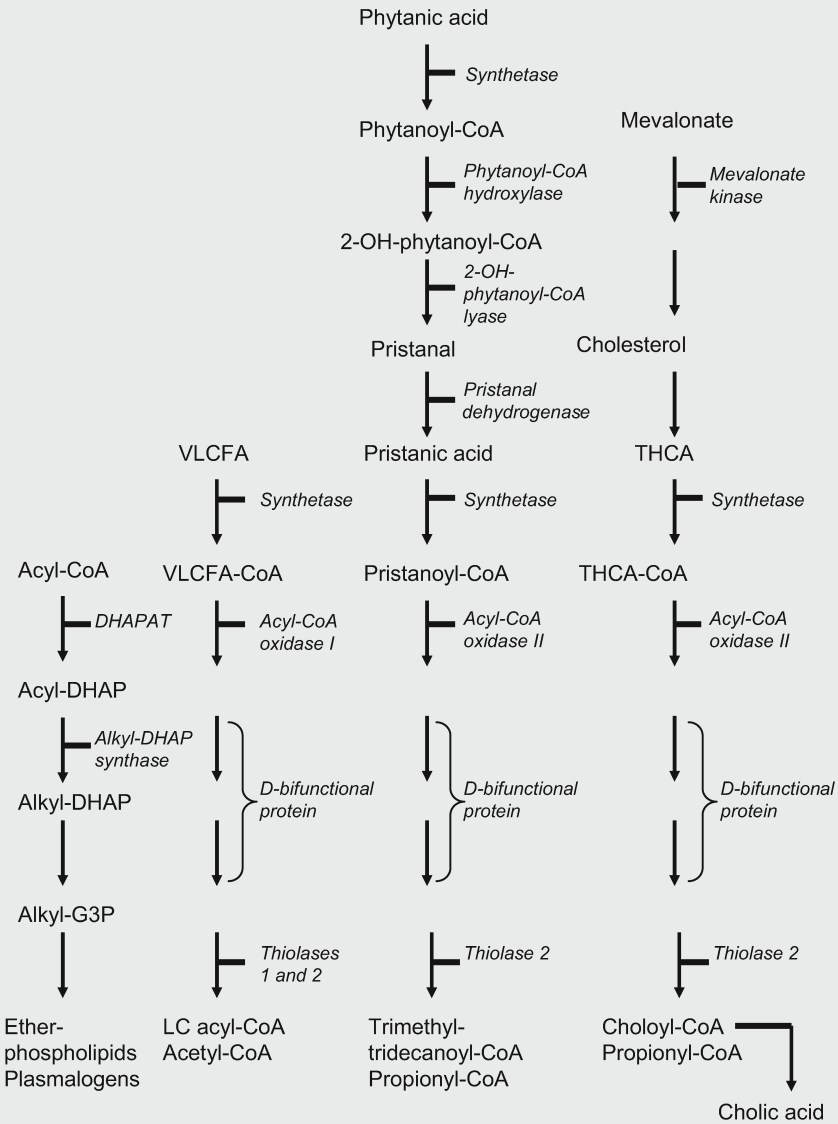
41

Peroxisomal Functions

Peroxisomes are cell organelles which derive their name from the presence of catalase, an enzyme that converts hydrogen peroxide into oxygen and water. Like lysosomes, they are found in all human cells except erythrocytes; however, unlike lysosomes, they possess anabolic as well as catabolic functions (◘ Fig. 41.1). Peroxisomes are mainly involved in lipid metabolism: they synthesise ether-phospholipids, called plasmalogens, which are important constituents of cell membranes and of myelin, and β-oxidise very-long-chain fatty acids. Peroxisomes are also involved in the oxidation of phytanic acid, a chlorophyll derivative, and in the formation of bile acids from mevalonate via cholesterol. In addition, they are concerned with the catabolism of lysine via pipecolic acid and glutaric acid (◘ Fig. 23.1), and of glyoxylate (◘ Fig. 43.1).



◘ **Fig. 41.1.** Schematic representation of the main peroxisomal functions. *CoA,* Coenzyme A; *DHAP,* dihydroxyacetone phosphate; *DHAPAT,* dihydroxyacetone phosphate acyltransferase; *G3P*, glycerol-3-phosphate; *LC*, long-chain; *THCA,* trihydroxycholestanoic acid; *VLCFA,* very-long-chain fatty acid

Peroxisomal disorders can be recognised by the presence of dysmorphic features, neurological abnormalities and hepatic and gastrointestinal dysfunction. Widely different features that can occur include:

- Craniofacial dysmorphism, skeletal abnormalities, shortened proximal limbs, calcific stippling of the epiphyses
- Encephalopathy, seizures, peripheral neuropathy, abnormal gait, hypotonia
- Ocular abnormalities such as retinopathy, cataracts
- Liver disease with hyperbilirubinaemia, hepatomegaly and cholestasis
- Failure to thrive
- Possibilities for (dietary) treatment are limited.

41.1 Clinical Presentation

At least 16 different disorders with marked clinical as well as biochemical heterogeneity linked to peroxisomal dysfunction have been identified [1-4]. In general, the onset of symptoms is not accompanied by an acute event or by abnormal routine laboratory tests indicating metabolic derangement. Most often, the presentation is associated either with variable neurodevelopment delay beginning in infancy or early childhood or with progressive neurological manifestations starting in the school-age period. Given the diversity of the clinical and biochemical abnormalities present in peroxisomal disorders, it is better to regard the clinical diagnosis as a function of both the presence of one or more of the following predominant features: dysmorphism, neurological dysfunction, and liver disease [5], and the age of the patient (◘ Table 41.1).

▪ Dysmorphism

Dysmorphic features, including large fontanelles, a high forehead, epicanthic folds and abnormal ears, may be mistaken for chromosomal disorders such as Down syndrome. Other abnormalities include: rhizomelic shortening of limbs in rhizomelic chondrodysplasia punctata (RCDP), calcific stippling of epiphyses in RCDP and

◘ **Table 41.1.** Clinical symptoms of peroxisomal disorders related to age

Symptoms	Disorder
Neonatal period	
Hypotonia, poor reactivity, seizures Craniofacial dysmorphism Skeletal abnormalities Conjugated hyperbilirubinaemia Increased lactate in CSF, blood	CZ, ZeS Acyl-CoA oxidase deficiency D-BP deficiency RCDP (typical/atypical) DLP1 deficiency
First 6 months	
Failure to thrive Hepatomegaly, prolonged jaundice Gastrointestinal problems, Hypocholesterolaemia Vitamin-E deficiency Visual abnormalities	ZeS D-BP deficiency Milder forms of RCDP
6 months to 4 years	
Failure to thrive Neurological presentation Psychomotor retardation Visual and hearing impairment (ERG, VEP, BAEP) Osteoporosis	ZeS Late-onset white matter disease Milder forms of RCDP
Beyond 4 years	
Behaviour changes, cognitive deterioration White-matter demyelination; spastic paraparesis Visual and hearing impairment Peripheral neuropathy, gait abnormality Ovarian dysgenesis	X-Linked ALD/AMN Classic Refsum disease Racemase deficiency PEX10 mutation Perrault syndrome with D-BP deficiency (HSD17B4), at least in some patients.

ALD, Adrenoleukodystrophy; AMN, adrenomyeloneuropathy; *BAEP*, brain auditory-evoked potentials; *CSF*, cerebrospinal fluid; *ERG*, electroretinogram; *RCDP*, rhizomelic chondrodysplasia punctata; *VEP*, visual-evoked potentials; ZeS, Zellweger spectrum; *CZ*, classic Zellweger; *D-BP*, D-bifunctional protein; *HSD17B4*,17β-hydroxy-steroid dehydrogenase type 4; *DLP1*, dynamin-like protein 1

classic Zellweger syndrome (CZ) and renal cysts in CZ. These congenital manifestations are indicative of dysmorphogenesis during the prenatal period [6].

▪ Neurological Dysfunction

In the neonatal period, the predominant symptoms often include severe hypotonia with loss of reactivity, which can be mistaken for a neuromuscular disease. Abnormalities in neuronal migration (neocortical dysplasia) and cerebral/cerebellar white matter disease may be present in CZ, neonatal adrenoleukodystrophy (NALD) and D-bifunctional protein (D-BP) deficiency. All disorders with an abnormal peroxisomal lipid metabolism present with neurological dysfunction of varying severity. An increasing number of inborn errors of metabolism without evident biochemical abnormalities on routine laboratory screening should also be considered in the diagnosis [6].

▪ Hepatic and Gastrointestinal Disease

The predominant manifestations may be hepatomegaly, cholestasis, hyperbilirubinaemia, prolonged jaundice, osteoporosis and failure to thrive, especially in disorders with a generalised deficiency of peroxisomal enzymes.

41.1.1 The Neonatal Period

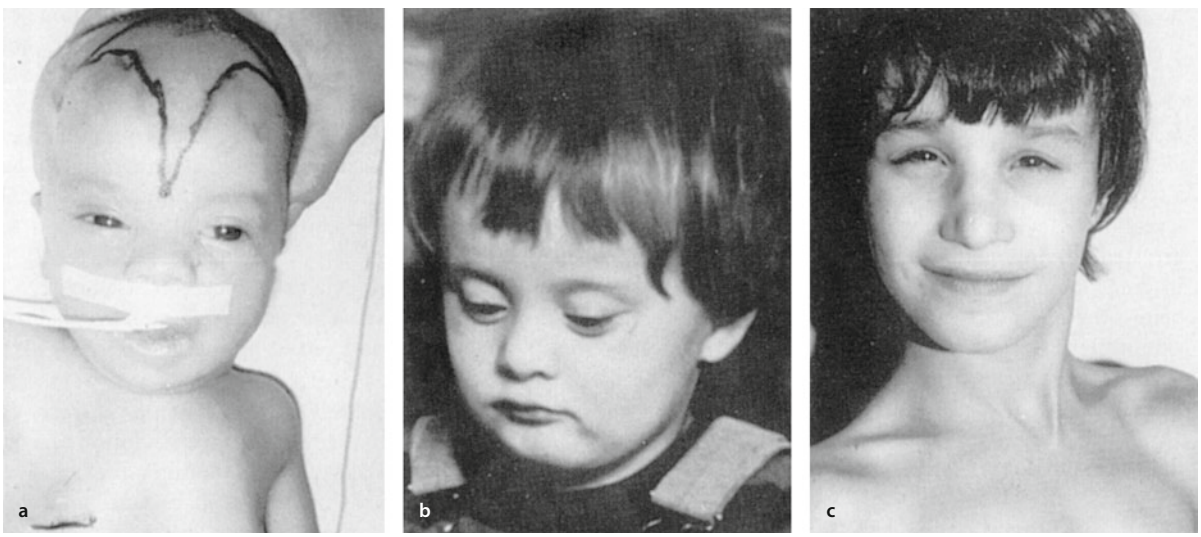
Two prototypes of peroxisomal disorders with a neonatal presentation are CZ syndrome, the most severe condition, and RCDP. Their phenotypes are distinct from that of the other peroxisomal disorders and should not cause difficulties in the differential diagnosis. Patients with NALD, D-BP deficiency and mevalonic aciduria may also have prominent manifestations in the neonatal period. The more recently described disorder of dynamin-like protein 1 (DLP1), which is required for mitochondrial and peroxisomal fission, also presents in the neonatal period with dysmorphism and severe neurological manifestations [7].

▪ Classic Zellweger Syndrome

Classic Zellweger syndrome (CZ) is characterised by:

- Errors of morphogenesis
- Neuronal migration disorder, germinolytic cysts, delay of myelination
- Severe hypotonia and weakness from birth
- Seizures
- Sensorineural deafness
- Ocular abnormalities
- Degenerative changes
- Liver disease
- Failure to thrive
- Absence of recognisable hepatic peroxisomes (presence of peroxisomal ghosts)
- Death in the 1st year

Infants with CZ display such characteristic physical features that the diagnosis can usually be made on examination alone (◘ Fig. 41.2a). Typically they show profound hypotonia and weakness, a severe swallowing disorder, very large fontanelles, eye abnormalities (pigmentary retinopathy, cataracts), seizures and hepatomegaly [3].



◘ **Fig. 41.2 a-c.** Three patients with multiple enzyme defects and defective peroxisome assembly: **a** classic Zellweger syndrome at 2 weeks of age; **b** infantile Refsum disease at 2 years of age (note facial dysmorphism resembling Down syndrome); **c** infantile Refsum disease at 11 years of age

▪ Classic Rhizomelic Chondrodysplasia Punctata

Classic RCDP is characterised clinically by the presence of severe proximal shortening of limbs, typical facial dysmorphism, cataracts, calcific stippling of epiphyses, coronal clefts of vertebral bodies, multiple joint contractures, and severe growth and psychomotor delays [3].

The calcific stippling may disappear after the age of 2 years. The chondrodysplasia punctata is more widespread than in CZ and may involve extraskeletal tissues. Some patients have ichthyosis. The classic RCDP phenotype is genetically heterogeneous, involving not only the *PEX7* gene (RCDP type 1) but also two other genes, *GNPAT* and *AGPS*, which code for the two peroxisomal enzymes involved in plasmalogen synthesis (RCDP type 2 and type 3). These three types are clinically indistinguishable from each other. Conversely, patients may have a *PEX7* gene deficiency and present a milder clinical phenotype without significant shortening of the long bones, much milder developmental delay, ability to walk and prolonged survival [8–11]. Classic RCDP and its milder variants must be distinguished from other forms of chondrodysplasia punctata, such as the autosomal dominant Conradi-Hünermann form with normal intelligence (▶ Chapter 33), the X-linked recessive form, and the X-linked dominant form (lethal in males).

▪ Neonatal Adrenoleukodystrophy

Patients with NALD are somewhat less severely affected than those with CZ [3]; dysmorphic features are less striking and may even be absent. The large majority present with a neonatal or early infantile onset of hypotonia and seizures. They have progressive white matter disease and may have pachypolymicrogyria; most die in late infancy. Calcific stippling of epiphyses and renal cysts are absent. Some patients can mimic a neuromuscular disorder resembling spinal muscular atrophy (Werdnig-Hoffman disease) [12]. Patients with a single peroxisomal β-oxidation enzyme defect at the level of straight-chain fatty acyl-coenzyme A oxidase [13, 14], but with clinical manifestations resembling those of NALD, have been described in the literature. Liver peroxisomes are normal or appear to be enlarged in size, whereas in CZ and NALD they are morphologically absent or severely decreased in number.

▪ D-Bifunctional Protein Deficiency

D-Bifunctional protein (D-BP) deficiency is the most frequently diagnosed single enzyme defect of peroxisomal fatty acid β-oxidation that mimics the clinical manifestations of CZ or NALD. Patients show severe nervous system involvement with hypotonia, seizures, absence of significant development and peripheral neuropathy [15-17]. The majority of patients with D-BP deficiency have a neuronal migration defect and progressive white matter disease [18, 19].

Dysmorphic features may be present: prominent forehead, flat nasal bridge and micrognathia. Most of the patients have died between the ages of 6 months and 2 years.

41.1.2 The First 6 Months of Life

During this period of life, the predominant symptoms may be hepatomegaly associated (or not associated) with prolonged jaundice, liver failure and nonspecific gastrointestinal problems (anorexia, vomiting, diarrhoea), leading to failure to thrive and osteoporosis. Hypocholesterolaemia, hypolipoproteinaemia and decreased levels of fat-soluble vitamins, symptoms that resemble a malabsorption syndrome, are frequently present (◘ Table 41.1). These patients can be erroneously diagnosed as having a congenital defect of glycosylation or a defect in the mitochondrial respiratory chain. Most CZ patients develop hepatomegaly and seizures and do not survive beyond this period. There is clinical, biochemical and genetic overlap among the three phenotypes, CZ, NALD and infantile Refsum disease (IRD) [3, 20], and the name Zellweger spectrum has been proposed for the whole group [21].

▪ Infantile Refsum Disease

IRD is the least severe phenotype within the group of peroxisome biogenesis disorders with generalised peroxisomal dysfunction. IRD patients have a later onset of variable initial symptoms, no neuronal migration disorder and no progressive white matter disease; their survival is variable. They may have external features reminiscent of CZ or little or no facial dysmorphism (◘ Fig. 41.2b,c). Their cognitive and motor development varies between severe global handicap and moderate learning disorder with deafness and visual impairment due to retinopathy. Many patients show growth failure and hyperoxaluria [20-22]. Cerebral findings on magnetic resonance imaging (MRI) are variable and differ from those of CZ in the predominance of regressive over developmental changes [23]. Most patients with IRD survive infancy, and some even reach adulthood.

41.1.3 Between 6 Months and 4 Years

During this period of life, cognitive and motor dysfunction becomes evident (◘ Table 41.1). Sensorineural hearing impairment is associated with abnormal brain stem auditory evoked responses. Various ocular abnormalities can be observed, including retinopathy, cataracts, optic nerve atrophy, glaucoma and brushfield spots. The

electroretinogram and visual evoked responses are frequently disturbed, and this may precede the fundoscopic abnormalities. Retinopathy associated with hearing loss, developmental delay and dysmorphism may be mistaken for other diseases, including malformative syndromes [24]. In this respect, the boundaries between malformative syndromes and inborn errors are not well delineated. Most CZ and NALD patients do not survive this period.

- **Late-onset White Matter Disease**

Late-onset cerebral white matter disease/leukodystrophy has been described in patients with a Zellweger spectrum phenotype, either following IRD or following normal early development and in the absence of distinct external features [25]. Two patients without facial dysmorphism had normal neurodevelopmental milestones during their 1st year, followed by rapid deterioration including severe hypotonia, seizures, retinopathy and deafness. A third patient initially diagnosed with IRD developed cerebral white matter degeneration in the 3rd year of life. MRI in all three patients revealed cerebral demyelination with sparing of subcortical fibres and pronounced central cerebellar demyelination.

41.1.4 Beyond 4 Years of Age

- **X-Linked Adrenoleukodystrophy/ Adrenomyeloneuropathy**

This is the most common peroxisomal disorder (1/20,000 males and females). The clinical picture can vary considerably even within the same family [26]. 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 (40% of all ALD phenotypes), with onset of neurological symptoms between 5 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 60% of adult ALD male patients (20-50 years) and 60% of heterozygous women (>40 years) [27]. The presentation in both sexes is with progressive spastic paraparesis. Subsequently, 35% of men with AMN develop cerebral demyelination, which, although it initially progresses more slowly than in boys, has ultimately the same fatal prognosis. Women with AMN do not develop cerebral demyelination.

The percentage of de novo *ALD* gene mutations is less than 6%, and consequently it is important to undertake genetic counselling and screening in ALD families in order to detect individuals at risk, including heterozygous women, boys with adrenal insufficiency and those who are asymptomatic with normal neuroimaging.

- **Classic Refsum Disease**

Classic Refsum disease (CRD) is another peroxisomal disorder with clinical onset during the school-age or adolescent period. Retinopathy, peripheral neuropathy, cerebellar ataxia and elevated CSF protein level without an increased number of cells are the main features. Less constant findings are sensorineural hearing loss, anosmia, skin changes, and skeletal and cardiac abnormalities [28, 29]. Mental retardation, liver dysfunction and dysmorphism are absent. The onset of symptoms is typically in late childhood or adolescence, but may be as late as the 5th decade. Without treatment patients show a gradually progressive deterioration.

Recently, patients with atypical Refsum disease have been reported with a defect in the peroxisomal fatty acid β-oxidation pathway, α-methyl-CoA racemase (AMACR) deficiency [30]. The clinical manifestations of racemase deficiency are those of an adult-onset sensory motor neuropathy and retinopathy.

Some patients with peroxisomal assembly defects can also present with an isolated motor and sensory peripheral neuropathy initially mimicking Charcot-Marie-Tooth disease [5], or with progressive ataxia, axonal motor neuropathy and cerebellar atrophy [31].

Although D-BP deficiency is generally a severe disease mimicking the Zellweger spectrum disorders, two patients (siblings) with Perrault syndrome, as characterised by ovarian dysgenesis, hearing loss and ataxia, have been described in the literature with mutations in the *D-BP* (*HSD17B4*) gene [32]. However, other patients with Perrault syndrome have no mutations in the *HSD17B4* gene, indicating that Perrault syndrome is genetically heterogeneous.

▪ Primary Hyperoxaluria Type 1

Primary hyperoxaluria type 1 (PH1), caused by deficiency or mistargeting of alanine: glyoxylate aminotransferase (AGT) in liver peroxisomes, is the most common and most severe form of the primary hyperoxalurias [33]. The clinical presentation of PH1 is very heterogeneous, with onset of symptoms between the neonatal period and 60 years of age. The most frequent form presents with recurrent urolithiasis and progressive renal failure leading to a diagnosis of PH1 in childhood or adolescence.

Extrarenal involvement includes the skeleton, leading to oxalate osteopathy, heart (cardiomyopathy, arrhythmias and heart block) nerves (polyradiculoneuropathy), joints (synovitis, chondrocalcinosis), skin (calcium oxalate nodules, livedo reticularis), soft tissues (peripheral gangrene), retina (retinopathy) and other visceral lesions (e.g. intestinal infarction, hypothyroidism).

Patients with a Zellweger spectrum disorder may also have hyperoxaluria [22].

41.2 Metabolic Derangements

The metabolic abnormalities observed in the different peroxisomal disorders (PDs) result either from defects in peroxisome biogenesis or from deficiencies of a single peroxisomal enzyme. They are listed in ◘ Table 41.2 and Table 41.3. Discussion of the different genetic defects is preceded by a brief review of the principal features of biogenesis and the metabolic function of peroxisomes (see ► [34] for a review).

◘ **Table 41.2.** Classification of peroxisomal disorders

No.	Disorder	Abbreviation	Protein involved	Gene(s)	Chromosome
Disorders of peroxisome biogenesis					
1	Zellweger syndrome	ZS	Different peroxins including: Pex1p,2p, 3p,5p,6p,10p,12p, 13p,14p,16p,19p,26p	PEX1, 2, 3, 5, 6, 10, 12, 13, 14, 16, 19, 26	Multiple loci
2	Neonatal adrenoleukodystrophy	NALD			
3	Infantile Refsum disease	IRD			
4	Rhizomelic chondrodysplasia punctata type 1	RCDP type 1	Pex7p	PEX7	6q22-924
Single peroxisomal enzyme deficiencies					
5	X-Linked adrenoleukodystrophy	X-ALD	ALDP	ABCD1	Xq28
6	Acyl-CoA oxidase deficiency (pseudo-neonatal ALD)	ACOX1-deficiency	Straight-chain acyl-CoA oxidase (SCOX/ACOX1)	ACOX1	17q25.1
7	D-Bifunctional protein deficiency	D-BP deficiency	D-BP/MF2/MFEII/D-PBE	HSD17B4	5q2
8	2-Methylacyl-CoA racemase deficiency	Racemase deficiency	AMACR	AMACR	5q13.2-p.12
9	Rhizomelic chondrodysplasia punctata type 2 (DHAPAT deficiency)	RCDP type 2	DHAPAT	GNPAT	1q42.1-42.3
10	Rhizomelic chondrodysplasia punctata type 3 (alkyl-DHAP synthase deficiency)	RCDP type 3	ADHAPS	AGPS	2q33
11	Refsum disease (phytanoyl-CoA hydroxylase deficiency)	ARD	Phytanoyl-CoA hydroxylase (PhyH)	PHYH / PAHX	10p15-p14
12	Hyperoxaluria type 1	PH1	Alanine glyoxylate aminotransferase (AGT)	AGXT	2q37.3
13	Glutaric acidaemia type 3	GA3	?	?	?
14	Acatalasia	-	Catalase	CAT	11p13
15	Mulibrey nanism	MUL	Trim37p	TRIM	17q22-23
16	Combined defect of mitochondrial and peroxisomal fission		Dynamin-like protein 1	DLP1	?

DHAPAT, dihydroxyacetonephosphate acyltransferase

Table 41.3. Biochemical characteristics of different peroxisomal disorders

	Diagnostic group								
	1			2			3	4	
	ZSDs (ZS, NALD, IRD)	AOXD	D-BPD	RCDP type 1	RCDP type 2	RCDP type 3	X-ALD	RD	AMACRD
Plasma									
Very-long-chain fatty acids	↑	↑	↑	N	N	N	↑	N	N
Di- and trihydroxy-cholestanoic acid	↑	N	↑[3]	N	N	N	N	N	↑
Phytanic acid	N-↑[1]	N	N-↑[4]	N-↑[2]	N	N	N	↑	N-↑
Pristanic acid	N-↑[2]	N	N-↑[c]	N	N	N	N	N	↑
Erythrocytes									
Plasmalogen level	↓	N	N	↓	↓	↓	N	N	N
Liver									
Peroxisomes	Deficient	Present, but abnormal	Present, but abnormal	Present	Present	Present	Present	N	NK
Fibroblasts									
Plasmalogen synthesis	↓	N	N	↓	↓	↓	N	N	N
DHAPAT	↓	N	N	↓	↓	↓[d]	N	N	N
Alkyl DHAP synthase	↓	N	N	↓	N	↓	N	N	N
C26:0 β-oxidation	↓	↓	↓	N	N	N	↓	N	N
Pristanic acid β-oxidation	↓	N	↓	N	N	N	N	N	↓
Acyl-CoA oxidase 1	↓	↓	N	N	N	N	N	N	N
D-Bifunctional protein	↓	N	↓	N	N	N	N	N	N
Phytanic acid α-oxidation	↓	N	N	↓	N	N	N	↓	N
Phytanoyl CoA hydroxylase	↓	N	N	↓	N	N	N	↓	N
Peroxisomes	Deficient	Present, but abnormal	Present, but abnormal	Present	Present	Present	Present	Present	Present
Mutant gene	PEX1, 2, 3, 5, 6, 10, 12, 13, 14, 16, 19, 26	ACOX1	HSD17B4	PEX7	GNPAT	AGPS	ABCD1	PAHX	AMACR

ZSDs, Zellweger spectrum disorders; *ZS,* Zellweger syndrome; *NALD,* neonatal adrenoleukodystrophy; *IRD,* infantile Refsum disease; *AOXD,* acyl-CoA oxidase 1 deficiency; *D-BPD,* D-bifunctional protein deficiency; *RCDP*, rhizomelic chondrodysplasia punctata; *X-ALD,* X-linked adrenoleukodystrophy; *RD,* Refsum disease; *AMACRD,* 2-methylacyl-CoA racemase deficiency; *N,* normal; *NK,* not known. [1]Phytanic acid is derived from dietary sources only and may therefore vary from normal to elevated in patients in whom phytanic acid α-oxidation is deficient. [2]Pristanic acid is derived from dietary sources only either directly or indirectly from phytanic acid via α-oxidation and may therefore vary from normal to elevated if pristanic acid β-oxidation is deficient. [3]Di- and trihydroxycholestanoic acid are not elevated in all D-BPD patients[15]. [4]Phytanic acid is often elevated if pristanic acid β-oxidation is impaired, even if phytanic acid β-oxidation per se is normal

41.2.1 Defects of Peroxisome Biogenesis

Normal Peroxisome Biogenesis

This process resembles the biogenesis of mitochondria in many respects. As with mitochondria, peroxisomes multiply by division of pre-existing peroxisomes. However, in contrast, peroxisomes lack their own DNA, all peroxisomal proteins being encoded by the nuclear genome. Peroxisomal proteins are synthesised on free polyribosomes and are specifically directed to peroxisomes via distinct peroxisomal targeting signals (PTS). This is true of both peroxisomal membrane and matrix proteins. Matrix proteins such as catalase, acyl-CoA oxidase and dihydroxyacetonephosphate acyltransferase (DHAPAT) contain one of two targeting signals (PTS1 and PTS2), which are small conformational units made up of a short stretch of amino acids. In the case of PTS1, the targeting information is contained in the last three carboxy terminal amino acids of a protein, whereas the PTS2 is determined by a stretch of nine amino acids. The PTS1 and PTS2 signals are recognised in the cytosol by specific receptors, the so-called PTS1 and PTS2 receptors, as encoded by the *PEX5* and *PEX7* genes, respectively. These two receptors carry the proteins specifically to the peroxisomal membrane, after which the peroxisomal protein import machinery transports the proteins across the peroxisomal membrane, where they catalyse their specific metabolic function. The peroxisomal protein import system is a multiprotein complex, and mutations in any gene coding for a component of this complex lead to a defect in peroxisome biogenesis. This implies that peroxisome biogenesis involves the correct expression of multiple so-called *PEX* genes, more than 15 of which have been identified in humans [34]. The corresponding proteins are called peroxins. These data explain the marked genetic heterogeneity among the peroxisome biogenesis disorders.

Peroxisome Biogenesis Defects

Within the group of peroxisome biogenesis defects a distinction must be made between the Zellweger spectrum disorders (ZSDs) on the one hand and RCDP type 1 on the other. In ZSDs, peroxisome biogenesis is fully defective, which results in a generalised loss of peroxisomal functions, as reflected in the following abnormalities: (1) impaired de novo plasmalogen biosynthesis, resulting in a generalised deficiency of plasmalogens in tissues including erythrocytes, (2) impaired peroxisomal β-oxidation, resulting in the accumulation of VLCFAs, pristanic acid, DHCA and THCA in plasma, and (3) deficient α-oxidation of phytanic acid, resulting in the accumulation of phytanic acid in plasma. Because phytanic acid and pristanic acid are derived from exogenous sources only, the accumulation of these compounds in ZSDs is dependent on both diet and age. In RCDP type 1 there is a selective defect in peroxisome biogenesis, affecting the PTS2 pathway only, whereas the PTS1 route is unimpaired. This implies that in RCDP most peroxisomal proteins are correctly targeted to peroxisomes, with the exception of the three PTS2 proteins, which include: (1) phytanoyl-CoA hydroxylase, (2) alkyl-DHAP synthase and (3) peroxisomal thiolase, with the ultimate consequence that plasmalogen biosynthesis and phytanic acid α-oxidation are deficient in RCDP type 1. This results in a deficiency of tissue plasmalogens and the accumulation of phytanic acid, again in an age- and diet-dependent manner. Peroxisomal β-oxidation, however, is completely normal in RCDP, which explains the normal plasma levels of VLCFAs, pristanic acid, and DHCA plus THCA (◘ Table 41.3).

In DLP1 deficiency there is a defect in the fission (division) of both peroxisomes and mitochondria, which share certain components involved in the fission process. This lethal disorder results in persistent elevation of plasma and CSF lactate, with a mild increase of VLCFA. Analysis of the mitochondrial respiratory chain complexes in a skeletal muscle biopsy reveals normal activities. Skin fibroblasts display elongated peroxisomes and mitochondria, but normal oxidative phosphorylation and peroxisomal beta oxidation [7].

41.2.2 Deficiencies of Single Peroxisomal Enzymes

Metabolic Functions of Peroxisomes

Peroxisomes catalyse a number of important metabolic functions (◘ Fig. 41.1). From the point of view of peroxisomal disorders the main peroxisomal functions in humans are: (1) fatty acid β-oxidation; (2) etherphospholipid biosynthesis; (3) fatty acid α-oxidation and (4) glyoxylate detoxification.

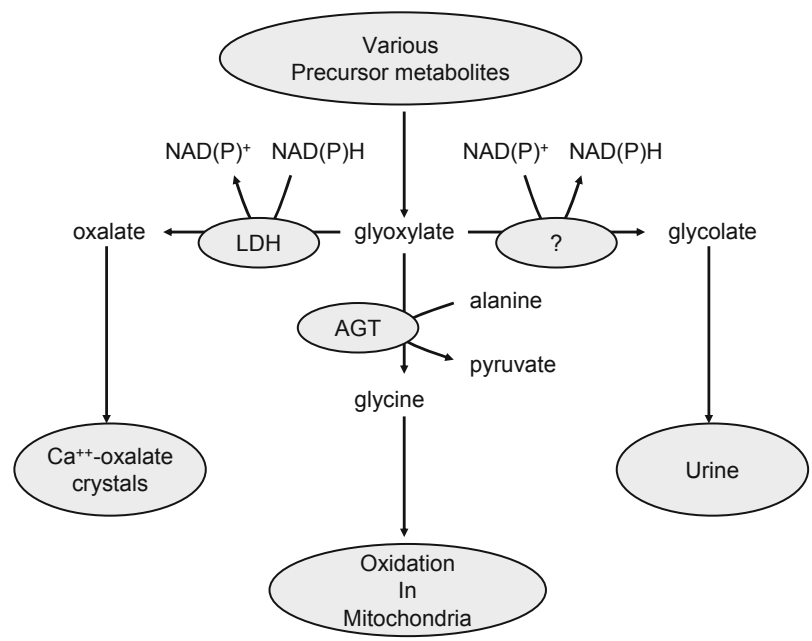
1. Fatty acid β-oxidation: Although most dietary fatty acids (FAs) are degraded in mitochondria, the peroxisomal β-oxidation system is of crucial importance since a number of FAs can only be oxidised by the peroxisomal system. These FAs include: (1) very long-chain FAs (VLCFAs); (2) pristanic acid and (3) di- and trihydroxycholestanoic acid (DHCA and THCA). The last two compounds are intermediates in the biosynthesis of bile acids, are formed from cholesterol in the liver and are β-oxidised within peroxisomes to produce chenodeoxycholic acid and cholic acid, respectively. In order to oxidise these different FAs, peroxisomes contain the enzymatic

machinery required to catalyse four consecutive steps of β-oxidation. The first step of peroxisomal β-oxidation is catalysed by one of two acyl-CoA oxidases, one of which is specific for straight-chain FAs, whereas the other is specific for branched-chain substrates. The second and third steps of peroxisomal β-oxidation are catalysed by a single protein with both hydratase and 3-hydroxyacyl-CoA dehydrogenase activities, named D-bifunctional protein (D-BP). Finally, two thiolases are involved in the fourth and last step of peroxisomal β-oxidation, one specific for straight-chain substrates and the other reactive with both straight-chain and branched-chain substrates. Degradation of some FAs requires the active participation of auxiliary enzymes, one of which, 2-methylacyl-CoA racemase (AMACR), plays an indispensable role in the degradation of pristanic acid and DHCA and THCA.

2. Etherphospholipid biosynthesis: Peroxisomes play an essential role in etherphospholipid biosynthesis, since the first two steps of the pathway, i.e. the formation of acyl-dihydroxyacetone phosphate (DHAP) and its subsequent conversion to alkyl-DHAP, are localised in peroxisomes. The enzymes involved are called dihydroxyacetonephosphate acyltransferase (DHAPAT) and alkyl-dihydroxyacetonephosphate synthase (alkyl-DHAP synthase or ADHAPS). The alkyl-DHAP, as synthesised in peroxisomes, is subsequently transported to the endoplasmic reticulum, where conversion into plasmalogens takes place.
3. Phytanic acid α-oxidation: In contrast to straight-chain FAs and 2-methyl branched-chain FAs, 3-methyl branched-chain FAs, such as phytanic acid, cannot undergo β-oxidation. Nature has solved this problem by creating a mechanism called α-oxidation, which allows oxidative removal of the terminal carboxyl group as CO_2 by 2-OH-phytanoyl-CoA lyase (◘ Fig. 41.1). This mechanism ensures that 3-methyl branched-chain FAs are chain-shortened into the 2-methyl FA, pristanal, which can then undergo regular β-oxidation [35].
4. Glyoxylate detoxification: Glyoxylate is a toxic compound since it is readily converted into oxalate, which precipitates in the presence of calcium ions. For this reason glyoxylate is rapidly detoxified by the liver-specific pyridoxal phosphate-dependent peroxisomal enzyme, alanine glyoxylate aminotransferase (◘ Fig. 41.3) [33, 36].

▪ Single Peroxisomal Enzyme Deficiencies

The single peroxisomal enzyme deficiencies can be subdivided into separate groups, based on the metabolic pathway affected. The disorders of peroxisomal β-oxidation include X-linked adrenoleukodystrophy (X-ALD), acyl-CoA oxidase deficiency, D-BP deficiency and 2-methylacyl-CoA racemase deficiency. In X-ALD there is a selective defect in the peroxisomal β-oxidation of VLCFAs but normal oxidation of the branched-chain fatty acids, pristanic acid, and DHCA and THCA. This is based on the notion that the protein defective in X-ALD,



◘ **Fig. 41.3.** Schematic representation depicting the detoxification of glyoxylate to glycine via the peroxisomal enzyme alanineglyoxylate aminotransferase. *LDH*, Lactate dehydrogenase; *AGT*, alanineglyoxylate aminotransferase

called ALDP, is involved in the uptake of VLCFAs across the peroxisomal membrane, but is not involved in the uptake of pristanic acid and DHCA and THCA. In D-BP deficiency, oxidation of all peroxisomal fatty acids is impaired, which follows logically from the fact that D-BP is the single enzyme protein involved in the oxidation of all peroxisomal fatty acids. This explains the accumulation of VLCFAs, pristanic acid, and DHCA and THCA in patients deficient in D-BP. It should be noted that three different subgroups of D-BP deficiency have been identified. In acyl-CoA oxidase deficiency the oxidation of VLCFA is affected, with normal oxidation of pristanic acid and DHCA and THCA. Finally, in 2-methylacyl-CoA racemase (AMACR) deficiency, only the peroxisomal β-oxidation of the branched-chain fatty acids (pristanic acid, and DHCA plus THCA) is impaired, with normal oxidation of VLCFAs, which explains the accumulation of pristanic acid and DHCA and THCA in patients with this disorder.

The group of plasmalogen biosynthesis defects includes (1) DHAPAT deficiency and (2) alkyl-DHAP synthase deficiency. In both cases plasmalogen biosynthesis is deficient. In contrast to RCDP type 1, which is due to mutations in the *PEX7* gene, in RCDP type 2 and type 3, where there are isolated deficiencies of plasmalogen biosynthesis, phytanic acid α-oxidation is unaffected, and as a consequence plasma phytanic acid levels are normal. Refsum disease is so far the only isolated disorder of phytanic acid α-oxidation. As a consequence of the deficient activity of the enzyme phytanoyl-CoA hydroxylase, phytanic acid accumulates in a time- and diet-dependent manner.

In hyperoxaluria type 1, where alanine glyoxylate aminotransferase is dysfunctional, there is an accumulation of glyoxylate, which is subsequently converted into glycolate and oxalate, a poorly soluble end-product (◘ Fig. 41.3). Deposits of calcium oxalate crystals in the kidney lead to stones, nephrocalcinosis and deteriorating kidney function, while bone disease is the most severe extrarenal involvement.

Finally, glutaric aciduria type 3 and acatalasia are rare disorders described in a few patients only.

41.3 Genetics

With the exception of X-ALD the pattern of inheritance of peroxisomal disorders is autosomal recessive. Zellweger spectrum disorders show a large degree of genetic heterogeneity (◘ Fig. 41.2) with 12 different genes currently implicated, including the following *PEX* genes: *PEX1*, *PEX2*, *PEX3*, *PEX5*, *PEX6*, *PEX10*, *PEX12*, *PEX13*, *PEX14, PEX16*, *PEX19* and *PEX26* [34]. Mutations in one particular *PEX* gene, i.e. *PEX7*, are associated with a different clinical phenotype, RCDP type 1 [37, 38]. As discussed above, RCDP is itself also genetically heterogeneous, since RCDP types 2 and 3 are caused by mutations in the *GNPAT* and *AGPS* genes coding for the first (DHAPAT) and second (alkyl-DHAP synthase) enzyme of plasmalogen biosynthesis, respectively. The other peroxisomal disorders do not show genetic heterogeneity, although it should be emphasised that mild mutations in the *PEX7* gene can give rise to a phenotype resembling Refsum disease rather than RCDP. Finally, Mulibrey nanism is caused by mutations in the gene *TRIM,* which codes for a protein named TRIM37, which is presumed to be localised in peroxisomes but has no known function in peroxisome biogenesis or peroxisome function [39].

41.4 Diagnostic Tests

Firstly, it is important to emphasise that there is no single laboratory test capable of identifying all peroxisomal disorders in a single analysis. Selection of the appropriate investigation(s) should be based on the clinical presentation. We have introduced the concept of diagnostic groups in order to develop logical guidelines for the laboratory diagnosis of the various disorders. These diagnostic groups include:

1. Zellweger spectrum disorders (ZS, NALD, IRD), acyl-CoA oxidase deficiency and D-BP deficiency.
2. Rhizomelic chondrodysplasia punctata spectrum disorders. This group includes all peroxisomal forms of RCDP, including types 1, 2 and 3.
3. X-Linked adrenoleukodystrophy complex. This embraces all types, including childhood cerebral ALD (CCALD) and AMN.
4. The remaining peroxisomal disorders. These include Refsum's disease, 2-methylacyl-CoA racemase (AMACR) deficiency, hyperoxaluria type 1, glutaryl-CoA oxidase deficiency, acatalasia and Mulibrey nanism.

41.4.1 Diagnostic Group 1

VLCFAs are abnormal in all disorders belonging to diagnostic group 1 [34] (◘ Table 41.3); normal levels exclude a ZSD or peroxisomal β-oxidation defect. If VLCFAs are abnormal, additional tests should then be performed to allow further discrimination. These include the analysis of plasmalogens in erythrocytes and bile acid intermediates, pristanic acid and phytanic acids in plasma. If

erythrocyte plasmalogens levels are deficient the patient is definitely suffering from a ZSD. Normal plasmalogen levels usually, but not always, point to a peroxisomal β-oxidation defect. These analyses should be followed by detailed studies in fibroblasts to establish whether there is a disorder of peroxisome biogenesis or an isolated β-oxidation disorder. In the case of ZSD complementation studies must be performed, with the ultimate aim of identifying the gene that is defective in the patient (► [34] for more details).

Although definitely not a first-line diagnostic test, pipecolic acid analysis has been found to be helpful in the identification of patients, especially since amino acid analysis is often done as part of a general screening programme for inborn errors of metabolism [40].

If fibroblast studies have shown an isolated peroxisomal β-oxidation defect, the activity of the different enzymes involved needs to be measured to establish whether the diagnosis is acyl-CoA oxidase deficiency or D-BP deficiency; this is then followed by subsequent molecular analysis.

41.4.2 Diagnostic Group 2

The clinical similarities of the three peroxisomal forms of RCDP warrant their inclusion in a single diagnostic group. Erythrocyte plasmalogens are always deficient, even in milder cases, making this a reliable initial laboratory test [9]. Abnormal results should be followed by detailed studies in fibroblasts for finer discrimination between types 1, 2 and 3 (see [34] for full details).

41.4.3 Diagnostic Group 3

Analysis of plasma VLCFAs is a reliable initial test to verify whether a patient is affected by X-ALD [41]. If the result is abnormal, one may proceed with fibroblast studies followed by molecular analysis. It should be noted, however, that fibroblast studies are not absolutely obligatory and direct molecular studies in blood cells can be performed too. Heterozygote detection is not as straightforward. Plasma VLCFAs have been found to be normal in about 5-15% of obligate heterozygotes. We advocate performing molecular studies and omitting VLCFA analysis in families in which the molecular defect has been established in the index patient. Where there is no family history plasma VLCFA analysis can be performed, followed where necessary by fibroblast studies including immunofluorescence using antibodies against the ALD protein. This method may be especially rewarding, since the product of the mutant X-ALD allele often produces an unstable protein, so that in heterozygotes a mosaic pattern is observed [42].

41.4.4 Diagnostic Group 4

- **Refsum Disease**

Plasma phytanic acid levels, although varying widely in patients with Refsum disease, are always abnormal and therefore a reliable indicator of the disease. A definite diagnosis requires measurements of phytanoyl-CoA hydroxylase in fibroblasts followed by molecular analyses. In a subset of patients no mutations are found in the hydroxylase gene. Studies have shown that in these patients there is a mutation in *PEX7* [43].

- **Primary Hyperoxaluria Type 1 (PH1)**

In most patients with PH1 there is increased urinary excretion of glyoxylate, oxalate and glycolate. Until recently, a definitive diagnosis of PH1 was thought to require assessment of AGT activity in liver. However, AGT activity may not be deficient in a large group of patients in which the enzyme is mistargeted to the mitochondria. Consequently, the current consensus is that molecular analysis of the *AGT* gene is the preferred method of definitively establishing a diagnosis. There is a high incidence of hyperoxaluria in Zellweger spectrum disorders [22].

- **Peroxisomal 2-Methylacyl-CoA Racemase (AMACR) Deficiency**

Patients with a deficiency of AMACR are unable to degrade pristanic acid and the bile acid intermediates. Plasma studies should, therefore, include analysis of pristanic acid by GC-MS and bile acid intermediates, preferably by tandem-MS. If results are abnormal, fibroblast studies, including measurements of racemase activity, and molecular studies should be performed [30].

41.4.5 Histological Detection

The abundance, size and structure of liver peroxisomes can be studied using the diaminobenzidine (DAB) procedure (which reacts with the peroxisomal marker enzyme catalase) and immunocytochemical techniques with antibodies against matrix and membrane peroxisomal proteins [44]. When peroxisomes are lacking, virtually all of the catalase is present in the cytosolic fraction instead of the particulate fraction. The term ‘membrane ghosts’ refers to empty-looking vesicles surrounded by a membrane. These vesicles represent abortive peroxisomes,

which are unable to import their matrix enzyme proteins. In some patients with a Zellweger spectrum, a mosaic distribution of peroxisomes in the liver and in fibroblasts can be observed [45].

41.4.6 Prenatal Diagnosis

Prenatal diagnosis of all peroxisomal disorders is now feasible, usually using chorionic villus biopsy material, and there is a shift from biochemical to molecular methods. In X-ALD, prenatal diagnosis relies first on sex determination using the detection of Y chromosome in maternal blood. ALD male fetuses are then identified using chorionic villus samples by direct analysis of the ALD protein and/or *ALD* gene mutation. When the *ALD* gene mutation is not yet known in the index case, VLCFA can be measured on cultured chorionic-villus samples or amniocytes.

41.5 Treatment and Prognosis

In classic Refsum disease reduction of phytanic acid levels by a low-phytanate diet (especially prohibition of ruminant meats and fats), with or without plasmapheresis, has been successful in arresting the progress of the peripheral neuropathy. However, when the diet is too strict (less than 10 mg phytanate/day) it may lead to a reduction in the energy intake, weight loss and a paradoxical rise in plasma phytanic acid levels followed by clinical deterioration. This is due to the mobilisation of phytanate from lipids stored in adipose tissue.

In X-linked ALD patients, haematopoietic cell transplantation (HCT) can stabilise or even reverse cerebral demyelination when the procedure is performed at a very early stage [46], i.e. when patients have no evident neurological or neuropsychological symptoms. The indication of HCT relies in each case on careful clinical and neuroimaging analysis of disease progression and severity. In practice, less than 50% of patients who are candidates for HCT can receive transplants during the therapeutic window in which the procedure is efficacious. Autologous haematopoietic stem cell gene therapy with lentiviral vector may prove a valuable alternative, given the recent success obtained in two patients [47]. There is no treatment for the inflammatory cerebral form of ALD. Lorenzo's oil (a mixture of oleic and erucic acid) allows normalisation of plasma VLCFA but unfortunately has no curative or preventive effects, at least in symptomatic patients. Interestingly, Lorenzo's oil may be beneficial if administered to asymptomatic patients; Moser et al. [48] performed a prospective study on Lorenzo's oil in 89 asymptomatic boys with a mean (SD) follow-up of 6.9 (2.7) years and observed a significant reduction in the risk of developing MRI abnormalities. Follow-up with brain MRI every 6 months should be undertaken in all asymptomatic boys from the ages of 4 to 12 years, and then once a year up to 45 years of age, in order to detect early signs of cerebral demyelination. This will allow any early changes to be identified so that allogeneic HCT can be undertaken, provided an HLA-matched donor is available.

The ALD mouse model develops a late-onset AMN-like phenotype. Therapeutic strategies aimed at: (1) overexpression of the *ABCD2* gene which codes for the protein ALDRP with properties similar to those of ALDP; and (2) decreased oxidative stress, are currently being evaluated in the ALD mice [49].

For patients with abnormal peroxisomal assembly and defects that originate before birth, the possibilities for treatment are very limited. Attempts in these patients have included dietary measures, such as phytanic acid restriction, treatment of deficiencies of fat-soluble vitamins, especially vitamins E and K, decreasing the abnormal bile acids using cholic acid, preventing the formation of calcium oxalate [33] and Addison crises, and the correction of docosahexaenoic acid and alkylglycerol deficiency by oral supplements [50-52]. In view of its lack of efficacy treatment remains primarily supportive.

The treatment of primary hyperoxaluria type 1 should be started as soon as the diagnosis has been made. The aims are to decrease oxalate production and to increase the urinary solubility of calcium oxalate by high fluid intake, calcium-oxalate crystallisation inhibitors and pyridoxine [53].

Pyridoxine (co-factor AGT) sensitivity (>30% reduction of urinary oxalate excretion) is found in 10-30% of patients. Extracorporeal shock-wave lithotripsy may be an option in some patients. Currently, liver and kidney transplantation are the final options [54].

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Congenital Disorders of Glycosylation

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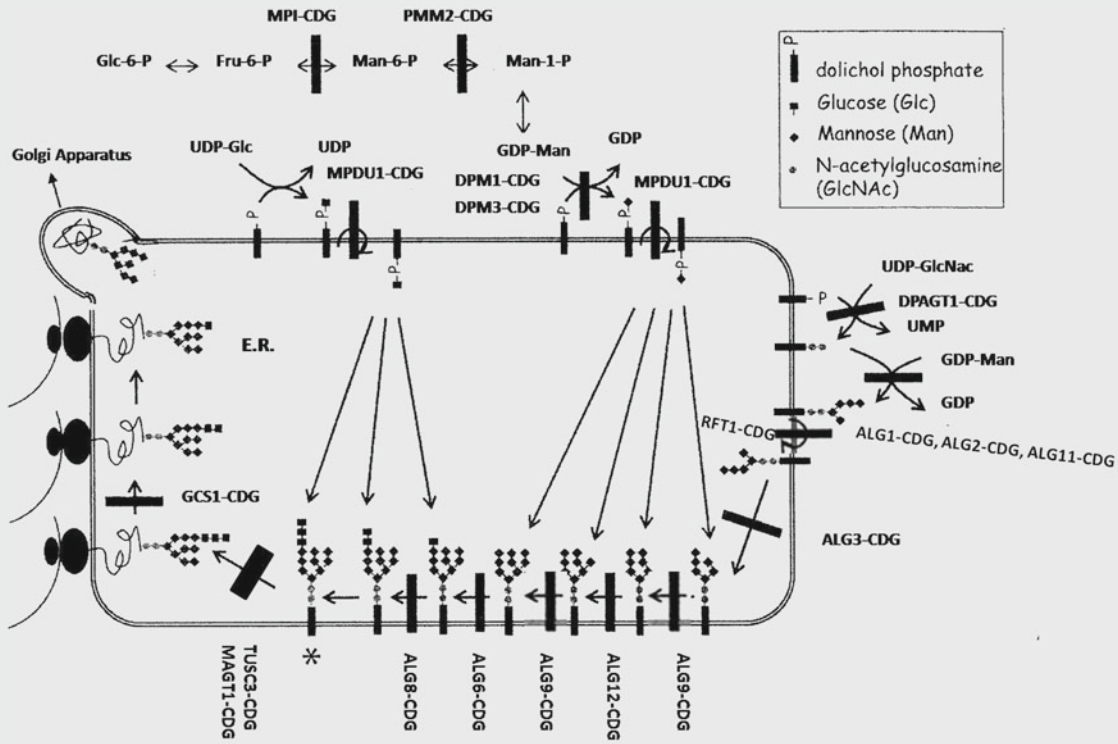
Synthesis of N-Glycans

This complex synthesis proceeds in three stages, schematically represented in ◘ Fig. 42.1.

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 of the 14-unit oligosaccharide precursor, dolichol pyrophosphate-N-acetylglucosamine$_2$-mannose$_9$-glucose$_3$ (indicated by an asterisk in the lower left part of ◘ Fig. 42.1).
3. Transfer of this precursor onto the nascent protein (depicted in the left part of ◘ Fig. 42.1), followed by final processing of the glycan in the Golgi apparatus by trimming and attachment of various sugar units.



◘ **Fig. 42.1.** Schematic representation of the synthesis of *N*-glycans. *ER*, endoplasmic reticulum; *Fru,* fructose; GDP, guanosine diphosphate; *Glc*, glucose; *GlcNAc*, *N*-acetylglucosamine; *Man*, mannose; *P*, phosphate; *UDP*, uridine diphosphate; *, dolichol pyrophosphate-*N*-acetylglucosamine$_2$-mannose$_9$-glucose$_3$. Defects are indicated by *solid bars* across the *arrows*. (Modified after [6])

42.1 Introduction

Numerous proteins are glycosylated with monosaccharides and/or oligosaccharide structures (◘ Fig. 42.1), 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. 42.1, 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

Table 42.1. Protein *N*-glycosylation disorders

Name	Main clinically affected organs and systems	Defective protein
PMM2-CDG (*CDG-Ia*)	Nervous system, fat tissue, other organs[1]	Phosphomannomutase 2
MPI-CDG (*CDG-Ib*)	Intestine, liver	Phosphomannose isomerase
ALG6-CDG (*CDG-Ic*)	Nervous system, intestine	Glucosyltransferase I
ALG3-CDG (*CDG-Id*)	Nervous system	Mannosyltransferase VI
ALG12-CDG (*CDG-Ig*)	Nervous system	Mannosyltransferase VIII
ALG8-CDG (*CDG-Ih*)	Intestine, liver	Glucosyltransferase II
ALG2-CDG (*CDG-Ii*)	Nervous system, eyes, liver	Mannosyltransferase II
DPAGT1-CDG (*CDG-Ij*)	Nervous system	UDP-GlcNAc: Dol-P-GlcNAc-P Transferase
ALG1-CDG (*CDG-Ik*)	Nervous system, liver	Mannosyltransferase I
ALG9-CDG (*CDG-IL*)	Nervous system, liver	Mannosyltransferase VII/IX
ALG11-CDG	Nervous system, hearing	Mannosyltransferase IV/V
RFT1-CDG (*CDG-In*)	Nervous system, hearing	Flippase of $Man_5GlcNAc_2$-PP-Dol
MGAT2-CDG (*CDG-IIa*)	Nervous system, skeleton, intestine, immune system, dysmorphism	*N*-Acetylglucosaminyltransferase II
GCS1-CDG (*CDG-IIb*)	Nervous system, dysmorphism	Glucosidase I
TUSC3-CDG	Nervous system	Oligosaccharyltransferase subunit
MAGT1-CDG	Nervous system	Oligosaccharyltransferase subunit

[1]Eyes, heart, liver, kidneys, skeleton, gonads, immune system

Golgi apparatus. It forms a diversity of structures, such as O-xylosylglycans, O-N-acetylgalactosaminylglycans, O-mannosylglycans and O-fucosylglycans. Besides protein glycosylation, lipid glycosylation also exists, e.g. glycosylation of ceramide, which is essential for the biosynthesis of gangliosides.

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 23 to 44) since the previous edition of this book. Most protein glycosylation disorders are due to defects in the *N*-glycosylation pathway (16 disorders; Table 42.1). Eight disorders of *O*-glycosylation have been identified (Table 42.2). A third group is that of the recently delineated defects in glycosphingolipid and glycosylphosphatidylinositol anchor glycosylation, with only three disorders (Table 42.3). The fourth group comprises defects in multiple glycosylation pathways and in other pathways (17 disorders; Table 42.4). The defect of oligosaccharide-chain processing that leads to deficiency of the mannose-6-phosphate recognition marker of the lysosomal enzymes and causes mucolipidosis II or III is discussed in ▶ Chapter 40. The deficiencies of the lysosomal enzymes that degrade the oligosaccharide side chains, and cause oligosaccharidoses, are also discussed in that chapter.

In 2008, a novel nomenclature for CDG was introduced because the then current nomenclature, using roman figures and arabic letters to name and classify *N*-glycosylation disorders (CDG-Ia, CDG-Ib …; CDG-IIa, CDG-IIb …), was considered too complex. Moreover, it is uninformative [2]. The novel nomenclature uses the official gene symbol (not in italics), followed by '-CDG' [3] (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. The novel nomenclature is used in this text, but with the former nomenclature in round brackets and in italics in the tables.

Patients with CDG show a very broad spectrum of clinical manifestations. CDG should therefore be consid-

Table 42.2. Protein *O*-glycosylation disorders

Name	Main clinically affected organs and systems	Defective protein
Defects in *O*-xylosylglycan synthesis		
EXT1/EXT2-CDG (multiple exostoses syndrome)	Cartilage	Glucuronyltransferase/*N*-acetyl-d-hexosaminyltransferase
B4GALT7-CDG (progeroid variant of Ehlers-Danlos syndrome)	Generalised rapid ageing	β-1,4-Galactosyltransferase 7
Defect in *O*-*N*-acetylgalactosaminylglycan synthesis		
GALNT3-CDG (familial tumoral calcinosis)	Skin, subcutaneous tissue, kidneys	ppGaNTase-T3[1]
Defect in *O*-xylosyl/*N*-acetylgalactosaminylglycan synthesis		
SLC35D1-CDG (Schneckenbecken dysplasia)	Chondrodyplasia (neonatal, lethal)	Solute carrier family 35 (UDP-glucuronic acid/UDP-*N*-acetylgalactosamine dual transporter), member D1
Defects in *O*-mannosylglycan synthesis		
POMT1/POMT2-CDG (congenital muscular dystrophy spectrum)	Nervous system, eyes, skeletal muscles	*O*-Mannosyltransferase 1
POMGNT1-CDG (congenital muscular dystrophy spectrum)	Nervous system, eyes, skeletal muscles	*O*-Mannosyl-β-1,2-*N*-acetylglucosaminyltransferase 1
Defects in *O*-fucosylglycan synthesis		
LFNG-CDG (spondylocostal dysostosis type 3)	Axial skeleton and associated muscles	*O*-Fucose-specific β-1,3-*N*-acetylglucosaminyltransferase
B3GALTL-CDG (Peters plus syndrome)	Brain, eyes, skeleton, other organs, dysmorphism	*O*-Fucose-specific β-1,3-glucosyltransferase

[1]UDP-*N*-acetyl-α-d-galactosamine: polypeptide *N*-acetylgalactosaminyltransferase 3

Table 42.3. Lipid glycosylation disorders

Name	Main clinically affected organs and systems	Defective protein
ST3GAL5-CDG (Amish infantile epilepsy)	Brain	Lactosylceramide α-2,3-sialyltransferase (GM3 synthase)
PIGM-CDG (glycosylphosphatidylinositol deficiency)	Brain, splanchnic veins	Phosphatidylinositolglycan, class M
PIGV-CDG (glycosylphosphatidylinositol deficiency)	Brain, skeleton	Phosphatidylinositolglycan, class V

ered in any unexplained clinical condition, particularly in multi-organ disease with neurological involvement. Isoelectrofocusing of serum transferrin is still the screening method of choice, but it is important to realise that it is able to detect only a limited number of CDGs, namely *N*-glycosylation disorders associated with sialic acid deficiency [4]. The (partial) deficiency of sialic acid in these forms of CDG causes one of two main types of cathodal shift (Fig. 42.2, ▶ Section 42.2.1). A type 1 pattern indicates an assembly disorder, and PMM2-CDG (CDG-Ia) and MPI-CDG (CDG-Ib) should be considered first. If these are excluded the next step is dolichol-linked glycan analysis, which will usually locate the site of the defect. A type 2 pattern indicates a disorder of processing; protein-linked glycan analysis should then be performed in an attempt to identify the defective step. In addition,

Table 42.4. Defects in multiple glycosylation pathways and in other pathways

Name	Main clinically affected organs and systems	Defective protein
DPM1-CDG (*CDG-Ie*)	Brain, eyes, dysmorphism	GDP-Man: Dol-P-mannosyltransferase (Dol-P-Man synthase 1)
DPM3-CDG	Skeletal muscles, cardiac muscle	GDP-Man: Dol-P-mannosyltransferase (Dol-P-Man synthase 3)
MPDU1-CDG (*CDG-If*)	Brain, skin	Lec35 (Man-P-Dol utilisation 1)
B4GALT1-CDG (CDG-IId)	Brain	B-1,4-Galactosyltransferase 1
GNE-CDG (hereditary inclusion body myopathy)	Skeletal muscles	UDP-GlcNAc epimerase/kinase
SLC35A1-CDG (*CDG-IIf*) (CMP-sialic acid transporter deficiency)	Blood platelets, neutrophils	CMP-sialic acid transporter
Defect in dolichol synthesis		
DK1-CDG (CDG-Im) SRD5A3-CDG	Nervous system, heart, skin, hair Brain, eyes, skeleton, skin	Dolichol kinase Steroid 5alpha-reductase type 3
Defects in Conserved Oligomeric Golgi (COG) complex		
COG7-CDG (CDG-IIe)	Nervous system, skin, liver, dysmorphism (hyperthermia episodes, early lethality)	Component of conserved oligomeric Golgi complex 7
COG1-CDG (*CDG-IIg*)	Nervous system, liver	Component of conserved oligomeric Golgi complex 1
COG8-CDG (CDG-IIh)	Nervous system, liver, dysmorphism	Component of conserved oligomeric Golgi complex 8
COG4-CDG (CDG-IIj)	Nervous system, dysmorphism	Component of conserved oligomeric Golgi complex 4
COG5-CDG COG6-CDG	Nervous system Nervous system (bleeding tendency)	Component of conserved oligomeric Golgi complex 5 Component of conserved oligomeric Golgi complex 6
Defect in vesicular ATPase		
ATP6V0A2-CDG (autosomal recessive cutis laxa type 2)	Brain, skin, skeleton, other organs, dysmorphism	Subunit A2 of the V0 domain of vesicular H^+-ATPase
Defect in COP II		
SEC23B-CDG (congenital dyserythropoietic anaemia type II)	Erythrocytes	COP II component SEC23B

isoelectrofocusing of serum apolipoprotein C-III (which is only O-glycosylated) can detect some *O*-glycosylation disorders [5].

Since about 1% of the human genome is involved in glycosylation, it is more than probable that the majority of CDGs have still to be discovered. We predict that these will also include diseases that are due to defects in organ-specific glycosylation (brain-CDG, kidney-CDG, etc.). Also, as has already been shown for hereditary multiple exostoses, such as Walker-Warburg syndrome and others, there is no doubt that known diseases with unknown aetiology will continue to be identified as CDGs.

Only the most frequently reported CDG in each group will be described in more detail in this chapter. For recent reviews see ► [3, 6-11], and for recent reports not covered by these reviews see ► [12-23b].

42.2 Congenital Disorders of Protein N-Glycosylation

42.2.1 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 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 retardation (IQ typically between 40 and 60), 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 and 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 psychomotor retardation). Patients often have an extraverted and happy appearance. Neurological investigations reveal (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) 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. 42.1). 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, cholinesterase etc.), lysosomal enzymes and membranous glycoproteins.

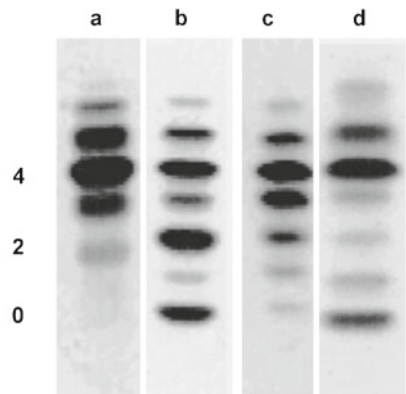
▪ Genetics

PMM deficiency is inherited as an autosomal-recessive trait caused by mutations of the *PMM2* gene on chromosome 16p13. At least 90 mutations (mainly missense) have been identified. The most frequent mutation causes an R141H substitution which, remarkably, has not yet been found in the homozygous state, pointing to a lethal condition. The frequency of this mutation in the normal Belgian population is as high as 1 in 50. The incidence of PMM 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 PMM deficiency and mutations in the *PMM2* gene. 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 PMM deficiency in particular) is usually made by isoelectrofocusing and immunofixation of serum transferrin (◘ Fig. 42.2). 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 recognised. Type 1 is characterised 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 the secondary glycosylation disorders, chronic alcoholism and hereditary fructose intolerance. A shift due



◘ **Fig. 42.2 a-d.** Serum transferrin isoelectrofocusing patterns. **a** Normal pattern; **b** type 1 pattern; **c**, **d** type 2 patterns; *0*, *2*, *4* each indicate the number of sialic acid residues

to a transferrin protein variant has first to be excluded (by isoelectrofocusing after neuraminidase treatment, study of another glycoprotein and 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 inconsiderable number of false-positive results. Recently, capillary zone electrophoresis of total serum has been introduced for the diagnosis of CDG.

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.

▪ Treatment and Prognosis

No effective treatment is available. The promising finding that mannose is able to correct glycosylation in fibroblasts with PMM deficiency could not be substantiated in patients. There is a substantially increased mortality (~20%) in the first years of life, which is due to severe infection or vital organ involvement (liver, cardiac or renal insufficiency) [24, 25].

42.2.2 Phosphomannose-Isomerase Deficiency (MPI-CDG)

▪ Clinical Presentation

Three groups independently reported this CDG first in 1998. Some 20 patients have been described. Most have presented with hepatic-intestinal disease without notable dysmorphism, and with only minor neurological involvement or none at all. 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. A healthy adult has been reported who had transient feeding problems in childhood. In the other patients, symptoms persisted and consisted 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; retrospectively they were shown most probably to have the same disease.

▪ Metabolic Derangement

Mannose-phosphate 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. 42.1). Hence the blood biochemical abnormalities are indistinguishable from those found in PMM2 deficiency. Since the substrate of MPI, fructose-6-phosphate, is efficiently metabolised in the glycolytic pathway, it does not accumulate intracellularly.

▪ Genetics

Inheritance of MPI deficiency is autosomal recessive. The gene has been localised to chromosome 15q22. Several mutations have been identified. Prenatal diagnosis is only possible if the molecular defect is known in the proband.

▪ Diagnostic Tests

Serum transferrin isoelectrofocusing 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 the corresponding gene.

▪ Treatment and Prognosis

MPI deficiency is the most rewarding CDG to diagnose because, so far, it is the only one known that can be effectively treated. Mannose is the therapeutic agent. 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 five doses) is used. The clinical symptoms usually disappear rapidly, but it takes several months before the transferrin isoelectrofocusing pattern improves significantly. However, in some patients this treatment cannot control the liver disease. One patient was unable to tolerate mannose. She was treated with heparin, which led to an improvement [26].

42.2.3 Glucosyltransferase I Deficiency (ALG6-CDG)

▪ Clinical Presentation

ALG6-CDG is the second most common *N*-glycosylation disorder, with more than 30 patients identified since its initial description in 1998. Clinical features in common with PMM2-CDG are hypotonia, strabismus and seizures, but psychomotor retardation is milder, in addition to which there is less dysmorphism and 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

Glucosyltransferase I deficiency 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 (Fig. 42.1). It causes hypoglycosylation of serum glycoproteins, because nonglucosylated 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 and protein C). That the clinical picture in these patients is much milder that that of PMM-deficient 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 the biosynthesis of glycosylphosphatidylinositol-anchored glycoproteins.

Genetics

Inheritance of this glucosyltransferase deficiency is autosomal recessive. The gene maps to chromosome 1p22.3. A333V is a common mutation. Prenatal diagnosis is only reliable if the molecular defect is known in the proband.

Diagnostic Tests

This disease illustrates that, even in cases of mild psychomotor retardation without any specific dysmorphic features, isoelectrofocusing 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 in fibroblasts. If the major fraction of these oligosaccharides consists of nine mannose and two *N*-acetylglucosamine residues without the three glucose residues that are normally present, this specific glucosyltransferase activity should be measured in fibroblasts (which is only undertaken by very few laboratories). If deficient activity is found then this should be followed by mutation analysis.

Treatment and Prognosis

No efficient treatment is available. The long-term outcome is unknown, since all reported patients have been children [27].

42.3 Congenital Disorders of Protein O-Glycosylation

42.3.1 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 *d*-glucuronic and *N*-acetylglucosamine units in the synthesis of heparan sulfate (Fig. 40.1). It has been hypothesised that mutations in these glycosyltransferases impair the synthesis of a glycosaminoglycan that exerts a tumour-suppression function. This would explain the higher risk of affected individuals to develop chondrosarcomas and osteosarcomas.

Mutations in *EXT1*, localised on chromosome 8q24.1, and in *EXT2,* on chromosome 11p11-p12, have been identified and are responsible for over 70% of cases of hereditary multiple exostoses. Prenatal diagnosis can be performed by mutation analysis [28].

42.3.2 Walker-Warburg Syndrome (POMT1/POMT2-CDG)

Walker-Warburg Syndrome is 1 of some 25 neuronal migration disorders known in humans. It is characterised by brain and eye dysgenesis associated with congenital muscular dystrophy. Male patients often have testicular defects. Psychomotor development is absent. 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, which catalyses the first step in the synthesis of the *O*-mannosylglycan core. It is caused by mutations in the gene *POMT1*, located on chromosome 9q34.1 [29].

42.3.3 Muscle-eye-brain Disease (POMGNT1-CDG)

Muscle-eye-brain disease is a neuronal migration/congenital muscular dystrophy syndrome similar to Walker-Warburg 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*, located on chromosome 1p34-p33 [29].

42.4 Defects in Lipid Glycosylation

42.4.1 GM3 Synthase Deficiency (ST3GAL5-CDG)

This first identified glycolipid glycosylation disorder was detected in an Old Order Amish pedigree. The first symptoms consisted of poor feeding and irritability appearing between 2 weeks and 3 months of age. Epilepsy developed within the 1st year (grand mal and other presentations) and was difficult to control. Moreover, the patients showed profound developmental stagnation with regression. On brain magnetic resonance imaging there was diffuse atrophy at an older age.

The metabolic derangement was identified as a defect of lactosylceramide α-2,3-sialyltransferase (also called GM3 synthase), which can be measured in plasma or fibroblasts. The defect causes accumulation of lactosylceramide associated with a decrease of the gangliosides of the GM3 and GD3 series. A homozygous mutation was found in *SIAT9*, localised on chromosome 2p11.2 [30].

42.5 Defects in Multiple Glycosylation Pathways and in Other Pathways

42.5.1 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 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 [31].

42.5.2 COG7 Deficiency

Seven patients have been reported with this CDG, all of North African origin. They had a severe syndrome consisting of feeding problems, growth retardation, dysmorphism, microcephaly, psychomotor retardation, hypotonia, cerebral atrophy, hyperthermia episodes, ventricular/atrial septum defects and cholestatic liver disease. Six of them died in their 1st year of life. The patients showed a type 2 pattern on serum transferrin isoelectrofocusing and an abnormal pattern of serum apolipoprotein C-III isoelectrofocusing. Studies of fibroblast glycoproteins showed a partial *N*- and *O*-glycosylation defect caused by a decreased transport of CMP-sialic acid and UDP-galactose into the Golgi, and a reduced activity of two glycosyltransferases involved in the galactosylation and sialylation of *O*-glycans. The six patients with early lethality were found to be homozygous for the same intronic mutation in *COG7*. This gene codes for subunit 7 of the Conserved Oligomeric Golgi (COG) complex, which is an eight-subunit peripheral Golgi membrane hetero-oligomeric protein complex. It is organised into lobes A (COG2-4) and B (COG5-7), with COG1 and COG8 bridging these lobes. Defects have also been discovered in COG1, COG4, COG5 and COG8, each in one or a few patients. 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' [32].

42.5.3 ATP6V0A2 (Autosomal Recessive Cutis Laxa Type 2)

Patients with this disorder, also called 'wrinkly skin syndrome' already have generalised 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 isoelectrofocusing pattern and an abnormal serum apolipoprotein C-III isoelectrofocusing 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. This seems to be another 'CDG-plus' [33].

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Cystinosis

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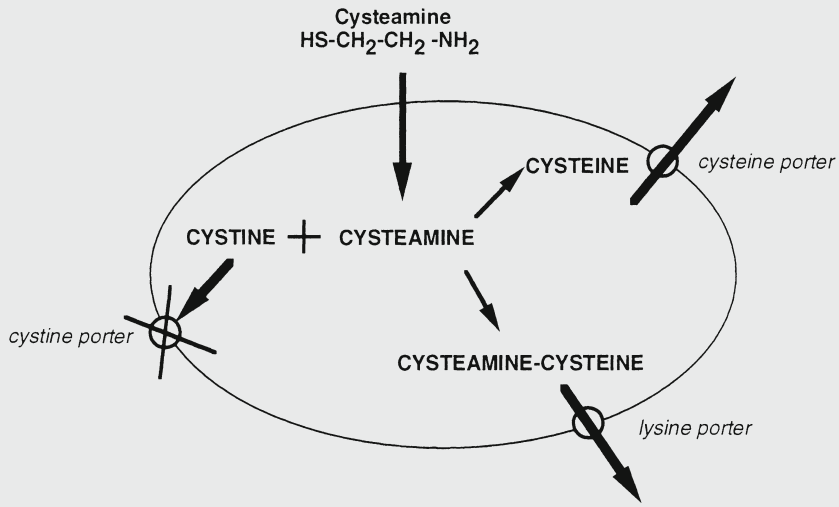
Lysosomal Porters for Cystine and Related Compounds

Intralysosomal cystine is formed by protein catabolism in the organelle, and is normally exported by a cystine porter (◘ Fig. 43.1) 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. 43.1.** Lysosomal export of cystine and related compounds

Cystinosis is a generalised lysosomal storage disease classified into three clinical phenotypes, of which the nephropathic or infantile form is by far the most frequent. The reported incidence of the disease is about 1 in 180,000 live births. The first symptoms start at about 6 months of age with anorexia, polyuria and failure to thrive and are secondary to a Fanconi proximal renal tubulopathy. In the absence of specific therapy, the renal disease progresses to end-stage renal disease when patients are between 6 and 12 years old. Survival beyond this age is associated with the development of extrarenal complications in eyes, thyroid, gonads, endocrine pancreas, muscle and central nervous system. An intermediate or juvenile-onset form, and a benign or adult form 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 the *CTNS* gene coding for *cystinosin*, a lysosomal carrier protein. 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.

43.1 Infantile Cystinosis

43.1.1 Clinical Presentation

- **First Stage**

The first 3-6 months of life are usually symptom-free. The first symptoms usually 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 with a peculiar odour, probably due to aminoaciduria, 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 normoglycaemic glycosuria, generalised aminoaciduria, tubular proteinuria (with massive excretion of β2-microglobulin and

lysozyme), decreased reabsorption of phosphate with hypophosphataemia, excessive losses of potassium, sodium and bicarbonate leading to hypokalaemia, hyponatraemia and metabolic acidosis. Hypercalciuria is also massive and hypouricaemia 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. Urolithiasis has been reported in rare cases, related to the high urinary excretion of urate, calcium and organic acids, and nephrocalcinosis may be observed [1]. Blond hair and a fair complexion with difficult tanning after exposure to the sun are often noted in white caucasian cystinotic children.

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. Confocal microscopy and optical coherence tomography allow 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. The pathological basis is a dramatic atrophy of the kidneys with glomerular sclerosis and tubulo-interstitial fibrosis. Early-onset renal failure without marked Fanconi syndrome has been reported [3]. 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. Repeat nasal bleeding is sometimes observed in cystinotic patients on dialysis [4]. After kidney transplantation there is no recurrence of the Fanconi syndrome even if cystine crystals are seen in the graft, located in monocytes and leukocytes.

▪ 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 emphasised the multisystemic nature of cystinosis, which may involve the eyes, thyroid, liver, spleen, pancreas, muscle and central nervous system (CNS) [4-7].

▪ Late Ocular Complications

The severity of ocular involvement differs from one patient to another [8]. 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 [9]. 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 [10]. Cataract has been reported [11].

▪ Endocrine Disturbances

Hypothyroidism. Thyroid dysfunction usually appears between 8 and 12 years of age, but it may be earlier or later. It is rarely overt with clinical symptoms, but rather discovered by systematic assessment of thyroid function [4]. Hypothyroidism may be partly responsible for growth impairment. Cysteamine was reported to delay or prevent thyroid dysfunction [12].

Gonadal Function. Abnormalities in the pituitary testicular axis, with a low plasma testosterone and high FSH/LH level [13], seem common in male patients with cystinosis. They may preclude full pubertal development. Male patients are generally azoospermic, but the presence of sperm in testicular biopsy may allow in vitro fertilisation [14]. Female patients exhibit pubertal delay but seem to have normal gonadal function, and there are several reports of successful pregnancies.

Endocrine Pancreas. Postoperative hyperglycaemia and permanent insulin-dependent diabetes have been reported in several series of cystinotic patients after kidney transplantation. Half of the patients not treated by cysteamine develop diabetes according to the WHO definition [15]. The exocrine pancreas is usually not affected, except in one reported case with steatorrhoea [16].

▪ Liver and Spleen Involvement

Hepatomegaly and splenomegaly occur in one-third to half of the cases who do not receive cysteamine after 15 years of age [4]. Hepatomegaly is related to enlarged Kupffer cells that transform into large foam cells contain-

ing cystine crystals. This enlargement may be the cause of portal hypertension with gastro-oesophageal varices. Cirrhosis has never been reported, but sclerosing cholangitis is possible [17]. Splenomegaly is also related to the development of foam cells in the red pulp. Haematological symptoms of hypersplenism may be noted. Cysteamine prevents complications of this type.

- **Muscle**

A distinctive 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 but with more severe involvement of the interosseous muscles and those of the thenar eminence [18, 19]. Pharyngeal and oral dysfunction, which may also cause voice changes, is often observed [20, 21]. Swallowing dysfunction is inversely related with the duration of cysteamine treatment [22]. Pulmonary dysfunction was recently reported in a series of adult nephropathic cystinotics up to 40 years of age; it was directly correlated with the severity of myopathy [23]. Nocturnal noninvasive positive pressure constitutes an effective treatment [24]. It is not clear whether a case of cardiomyopathy reported in a patient was directly related to cardiac cystine accumulation [25].

- **Central Nervous System**

Cystinosis does not affect general intellectual performances. Nevertheless, several neurological complications have been reported in cystinosis. Convulsions may occur at any age, but it is difficult to evaluate the direct responsibility of cystinosis in this complication when uraemia, electrolyte desequilibrium, drug toxicity etc. are present. Idiopathic intracranial hypertension was reported [26]. A subtle and specific visuoperceptual defect and lower cognitive performances with subtle impairment of visual memory and tactile recognition have been reported [27, 28], as have social difficulties [29]. 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 [30]. More severe CNS abnormalities with various defects have also been described [7, 31]. 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. In other cases, acute ischaemic episodes may occur with hemiplegia or aphasia. This cystinotic encephalopathy has only been observed in patients above 19 years of age, and at present it is difficult to know its actual incidence. The effectiveness of cysteamine treatment for the prevention of CNS involvement is not known either. Cysteamine treatment was associated in some cases with an improvement of neurological symptoms [31]. 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 15-20 years of age [31, 32].

43.1.2 Metabolic Derangement

Efflux of cystine out of cystinotic lysosomes is significantly decreased in comparison with normal lysosomes [33]. 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 [34, 35]. 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 [36] and inhibition of Na^+ dependent transporters [37]. Cellular cystine accumulation may also inhibit pyruvate kinase and creatine kinase activity in rat brain or pig retina [38-40]. Inhibition of adenylate cyclase activity by cystine in rat brain is prevented by cysteamine [41]. Cystine depletes the glutathione cell pool, thereby favouring oxidative stress and apoptosis [42, 43]. A knock-out mouse model lacking cystinosin was reported [44]. It will certainly be helpful for the understanding of the metabolic derangement and for therapeutic approaches.

43.1.3 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 seven membrane-spanning domains and two lysosomal targeting signals [45]. More than 50 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. In the other cases, point mutations or shorter deletions are found on both alleles, some of them clustering in certain ethnic and/or geographical areas [46].

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.

43.1.4 Diagnostic Tests

The diagnosis of cystinosis is confirmed by measurement of the free cystine content, usually in leukocytes, which in patients with nephropathic cystinosis is about 10-50 times normal values [47]. The assay, which uses a protein-binding technique on white blood cells, is very sensitive, and can be carried out on small 3-ml blood samples. In cystinosis, the level is usually 5-15 nmol of 1/2 cystine/mg protein. The technique enables detection of heterozygous carriers with levels of 0.5-1.4 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 [48]. The results obtained on polymorphonuclear leukocytes are approximately twice those obtained on mixed leukocytes, and this must be taken into account when comparing data. The measurements may also be carried out on fibroblasts, conjunctiva and muscle. *S*-Labelled cystine incorporation in cultured skin fibroblasts, amniotic cells, or chorionic villi enables a prenatal diagnosis to be made during the first trimester [40] The direct assay on chorionic villi cells may give the result within 24 h [49]. 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 [50]. At birth, diagnosis is possible on placenta or cord blood white cells.

43.1.5 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 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.
- **Sodium:** Sodium losses sometimes remain uncompensated after achievement of acid-base equilibrium. This is recognisable by a persistent hyponatraemia with failure to thrive.
- **Potassium:** Hypokalaemia requires potassium supplements in order to maintain serum potassium above 3 mmol/l; 4-10 mmol/kg is usually necessary to achieve this goal. Prescription of amiloride at a dose of 2-5 mg/day may help in some cases.
- **Phosphorus:** Hypophosphataemia 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 level just above 1.0-1.2 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** at a dose of 100 mg/kg per day in four divided doses has been proposed to correct muscle carnitine depletion [51].

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. Losses of water, potassium and sodium may be drastically reduced by the prescription of indomethacin at a dose of 1.5-3 mg/kg in two separate doses [42]. It has been shown that the angiotensin-converting enzyme (ACE) inhibitor, enalapril, diminishes albuminuria and possibly slows down the degradation of renal function [43]. When the glomerular filtration rate decreases, indomethacin must be stopped; at this time tubular losses also decrease, and the mineral supplements must be adjusted and progressively tapered off to avoid overload, especially with sodium and potassium. When dialysis is started, mineral supplements are usually no longer necessary.

Feeding problems may require tube or gastric button feeding, and in some cases continuous or intermittent total parenteral nutrition [52].

▪ Renal Replacement Therapy

There is no specific requirement for cystinotic children for this procedure at this stage. Haemodialysis and peri-

toneal dialysis are both effective and applied according to the circumstances. As for any child with ESRF, kidney transplantation is considered the best approach. Results of kidney transplantation in the European Dialysis and Transplant Association (EDTA) paediatric registry were better than for any other primary renal disease in children [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, was reported to be improved by administration of recombinant growth hormone at a dose of 1 U/kg/week [54]. Portal hypertension may lead to ascites and bleeding oesophageal varices, rendering a portal bypass necessary. 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. It has been shown that eye drops containing 0.5% cysteamine are able to prevent corneal deposits [9] and may decrease and even suppress the deposits already present. Corneal grafting has been performed only rarely, and with variable results.

▪ Specific Therapy

Several attempts have been made to suppress lysosomal cystine storage. Dietary restriction of sulfur amino acids has no effect. One drug, cysteamine ($HS\text{-}CH_2\text{-}NH_2$), has been tested in cystinotic patients with apparent benefit, as shown in a prospective study [55]. Cysteamine is commercially available as cysteamine bitartrate (Cystagon). The dose is progressively increased from 10 to 50 mg/kg of cysteamine base per day. Cysteamine 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 four separate doses – one every 6 h – 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 (mean daily dose of 28 mg/kg body weight) was as effective as the current formulation of cysteamine [57].

Careful monitoring of polymorphonuclear leukocyte cystine content is essential, since the response to cysteamine is variable. Polymorphonuclear leukocyte cystine content should be determined 6 h after the last dose and just prior the next: the aim is to keep cystine content under 2, or better under 1 nmol, of 1/2 cystine per mg of protein. The drug should be started as soon as the diagnosis is confirmed [58, 59]. The good results obtained in the treatment of the renal disease have encouraged the use of cysteamine in patients who are at risk of developing extrarenal complications. Side effects of the drug include nausea and vomiting and can be managed with omeprazole [60]. Less commonly, allergic rashes, seizures and neutropenia are seen. In addition, cysteamine is responsible for an unpleasant breath odour so that compliance with four doses per day is difficult to maintain in the long term, especially in adolescents [61].

A new therapeutic approach was tested on the animal model using bone marrow cell transplantation, with encouraging results [62].

43.2 Intermediate 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 its severity is sometimes in the nephrotic range. Fanconi syndrome may be absent or mild, and tubular losses are less important than in infantile cystinosis [63]. The same is true for extrarenal symptoms. ESRF may develop during adolescence or adult life.

The diagnosis is ascertained by the assessment of the cystine content of leukocytes. Genetic analysis shows homozygous or compound heterozygous mutations of the *CTNS* gene with at least one 'mild' mutation [64].

43.3 Ocular Cystinosis

Ocular (or adult or benign) cystinosis was first reported by Cogan et al. in 1957 [65]. This exceptional disorder is characterised by the presence of cystine crystals in the eye and the bone marrow [66]. 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 the *CTNS* gene that have been found in these patients encode a protein that allows sufficient residual cystine transport.

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Appendix A: Medications Used in the Treatment of Inborn Errors

JH Walter and JE Wraith

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Medication	Mode of action	Disorders	Recommended dose	Route	Remarks	Chapters
Agalsidase alfa (Replagal)	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		40
Agalsidase beta (Fabrazyme)	Recombinant analogue of human α-galactosidase A manufactured in Chinese Hamster Ovary (CHO) cell line	Fabry disease	1.0 mg/kg alt weeks	IV		40
Aglucosidase alfa (Myozyme®)	Recombinant analogue of human α-glucosidase manufactured in Chinese Hamster Ovary (CHO) cell line	Pompe disease	20 mg/kg alt weeks	IV		6
Allopurinol	Xanthine-oxidase inhibitor	Disorders leading to hyperuricaemia (PRPP synthetase superactivity; HGPRT deficiency) and APRT deficiency	Initial dosage 10-20 mg/kg per day in children and 2-10 mg/kg per day in adults	Oral		36
Ammonium tetrathiomolybdate	Chelating agent	Wilson's disease	160 mg/d in 6 divided doses	Oral		38
Betaine	Remethylates hct to meth	Classic homocystinuria Remethylation defects	100-150 mg/kg/day in two to three divided doses, max. dose 6-9 g/d	Oral		21
Biotin	Co-factor for carboxylases Treatment of presumed transporter defect	Biotinidase deficiency, Multiple carboxylase deficiency Biotin-responsive basal ganglia disease	5-20 mg/d	Oral or IV		27 2
Chenodeoxycholic acid	Inhibits cholesterol 7a-hydroxylase (rate-limiting enzyme in bile acid biosynthesis)	3β-Dehydrogenase def (3β-D); Δ^4-3-Oxosteroid 5β-reductase deficiency (3-ORD); Cerebrotendinous xanthomatosis (CTX)	3β-D: 12-18 mg/kg/d for first 2 months then 9-12 mg/kg/d; 3-ORD :8 mg/kg/day; CTX: 750 mg/day (adults)	Oral		34
Cholesterol	Replenishes cholesterol	Smith-Lemli-Opitz (SLO) syndrome	20-40 mg/kg/d in 3-4 divided doses	Oral		33
Cholestyramine	Bile acid sequestrant	Familial hypercholesterolaemia	Adults: 12-24 g /d children: (wt in kg/70 x adult dose) in four divided doses	Oral	Possible vitamin A, D, and K deficiency with prolonged treatment. Other bile acid resins include colestipol & colesevalam	32

Medication	Mode of action	Disorders	Recommended dose	Route	Remarks	Chapters
Cholic acid		Δ^4-3-Oxosteroid 5β-reductase deficiency (3-ORD)	8 mg/kg/day	Oral		33
Copper histidine	Increases intracellular copper	Menkes disease	100-200 µg Cu/d (newborn) 1 mg Cu/d in older children	SC		38
Creatine monohydrate	Replenishes creatine	Guanidinoacetate methyltransferase (GAMT) deficiency arginine:glycine amidinotransferase (AGAT) deficiency	300-400 mg/kg/d in three to six divided doses	Oral		16
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		36
Cysteamine/ phosphocysteamine	Depletes lysosomal cystine	Cystinosis	1.3 g/m^2/day of free-base), given every 6 h	Oral and eye drops	Phosphocysteamine more palatable	43
Dextromethorphan	NMDA channel antagonist	NKH	5-7 mg/kg/d in four divided doses	Oral	Doses up to 35/mg/d have been used	24
Diazoxide	Inhibits insulin secretion	Persistent hyperinsulinism	15 mg/kg/d (newborn); 10 mg/kg/d (infants), in three divided doses	Oral		10
Dichloroacetate	Stimulates PDH activity by inhibiting PDH kinase	Primary lactic acidosis	50 mg/kg/d in 3-4 divided doses	Oral	May cause polyneuropathy with prolonged use	12
Entacapone	Prevents the peripheral breakdown of l-dopa	Disorders of BH_4 synthesis	15mg/kg/d in two to three divided doses	Oral		17
Ezetimibe	Inhibits cholesterol absorption	Familial hypercholesterolaemia	10 mg/d	Oral		32
Folinic acid	Provides accessible source of folate for CNS	DHPR deficiency, UMP synthase deficiency (hereditary orotic aciduria), Methylene synthase deficiency, Methionine synthase deficiency, Hereditary folate malabsorption, Some disorders of cobalamin metabolism, Cerebral folate transporter	5-15 mg/d	Oral, IV		17, 28

Medication	Mode of action	Disorders	Recommended dose	Route	Remarks	Chapters
Galsulfase (Naglazyme)	Recombinant analogue of human *N*-acetylgalactosamine 4 sulfatase manufactured in Chinese Hamster Ovary (CHO) cell line	Mucopolysaccharidosis type VI	1.0 mg/kg per week	IV		40
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	32
G-CSF	Stimulates granulocyte production	Neutropenia in GSD Ib, Ic	5 μg/kg once daily	SC		6
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	19
Haem arginate	Inhibits 5-aminolevulinic acid synthase	Acute porphyrias	3-4 mg/kg once daily for 4 days	IV		37
Hydroxocobalamin (vitamin B_{12})	Co-factor for methylmalonyl mutase	Disorders of cobalamin metabolism	1 mg IM daily; oral dose 10 mg once or twice daily	IM or oral	Dose may be reduced to once or twice weekly according to response	19, 28
5-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	17,29
Idursulfase (Elaprase®)	Recombinant iduronate-2-sulfatase produced in human cell line	MPS II	0.5 mg per kg by IV infusion weekly	IV		40
Imiglucerase (Cerezyme)	Recombinant analogue of human β-glucocerebrosidase manufactured in Chinese Hamster Ovary (CHO) line	Gaucher disease	Various regimens: 2.5 U/kg 3X per week to 60 U/kg per 2 weeks For type III Gaucher disease some clinicians recommend higher dosages: 120 U/kg per 2 weeks	IV		40
Ketamine	*N*-Methyl-d-aspartate (NDMA) channel antagonist	NKH	1-30 mg/kg/d in four divided doses	Oral or IV		24
l–Arginine	Replenishes arginine; substrate of nitrous oxide	Urea cycle disorders; MELAS	50-170 mg/kg (OCT and CPS def) Up to 700 mg/kg in AL & AS def	Oral or IV	IV loading dose: (200 mg/kg) over 90 min	20

Medication	Mode of action	Disorders	Recommended dose	Route	Remarks	Chapters
Laronidase (Aldurazyme)	Recombinant analogue of human α-l-iduronidase manufactured in Chinese Hamster Ovary (CHO) line	Mucopolysaccharidosis type I	100 U/kg per week	IV		40
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	13, 19, ,23
l-Citrulline	Replenishes citrulline and arginine	Used as an alternative to arginine in CPS def and OCT def; LPI	CPS & OCT def: 170 mg/kg/day or 3.8 gm/m^2/day in divided doses, LPI: 100 mg/kg/d in 3-5 doses	Oral		20, 26
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	Allows lysine absorption	Lysinuric protein Intolerance	20-30 mg/kg/day in three divided doses	Oral		26
l-Serine	Replenishes serine	3-Phosphoglycerate dehydrogenase deficiency	up to 600 mg/d in sic divided doses	Oral		25
l-Tryptophan	Increases kynurenic acid which is an endogenous antagonist of the NMDA receptor	NKH	100 mg/kg/d in three divided doses	Oral		24
Magnesium	Replenishes Mg	Primary hypomagnesaemia with secondary hypocalcaemia	0.5-1.5 ml/kg/d $MgSO_4$ 10% solution IV; oral maintenance 0.7-3.5 mmol/kg/d elemental Mg in three to five divided doses	IV / oral		38
Mannose	Improves glycosylation	CDG Ib (PMI deficiency)	1 g /kg/d in five divided doses	Oral	Not of benefit in CDG Ia	42
Mercaptopropionylglycine (tiopronin)	Chelating agent	Cystinuria	15-20 mg/kg/d, up to max of 1,000 mg/d in three divided doses	Oral		26
Metranidazole	Reduces propionate production by gut bacteria	Propionic and methylmalonic acidaemia	7.5-20 mg/kg once daily	Oral		19
Miglustat (Zavesca)	Inhibitor of glucosylceramide synthase, the first enzyme responsible for glycosphingolipid (GSL) synthesis	Gaucher disease; neurological manifestations of NPC	100 mg TDS	Oral	Only recommended for patients with mild to moderate Gaucher disease who are unsuitable for enzyme replacement therapy.	39, 40

Medication	Mode of action	Disorders	Recommended dose	Route	Remarks	Chapters
N-Carbamoylglutamate	Stimulates *N*-acetylglutamate synthase	*N*-Acetylglutamate synthase deficiency, Carbamoylphosphate synthase deficiency Hyperammonaemia associated with organic acidaemias	100-300 mg/kg/day in four divided doses	Oral		19, 20
Nicotinamide	Replenishes deficiency state	Hartnup disease	50-300 mg/day	Oral		26
Nicotinic acid (niacin)	Inhibits the release of free fatty acids from adipose tissue; increases HDL-cholesterol	Hyperlipidaemia (see Chapter 32 for indications)	Adult dose: 100–200 mg 3 times daily, gradually increased over 2–4 weeks to 1–2 g three times daily	Oral		32
NTBC (2-[2-nitro-4-trifluoro-methylbenzoyl]-1,3-cyclhexanedione)	Inhibits 4-hydroxyphenylpyruvate dioxygenase	Tyrosinaemia type I	1 mg/kg in one to two divided doses	Oral	Combine with low-TYR, low-PHE diet to maintain plasma TYR<600 µmol/l	18
Octreotide	Somatostatin anaologue	Persistent hyperinsulinism	10 µg/d to 60 µg/d, given in three or four divided doses or by continuous pump	SC		10
Penicillamine	Chelating agent	Wilson disease; cystinuria	Wilson disease: up to 20 mg/kg/day in divided doses (min 500 mg/d); Cystinuria: 2 g/1.73 m^2	Oral		26, 38
Pyridoxine	Co-factor	Pyridoxine-responsive γ-cystathionase deficiency; Pyridoxine-responsive cystathionine β-synthase (CBS) deficiency; Pyridoxine dependency with seizures; pyridoxine-responsive OAT deficiency; X-linked sideroblastic anaemia; primary hyperoxaluria type 1	50-500 mg/d Pyridoxine dependency with seizures: 100 mg IV with EEG monitoring or 30 mg/kg/d for 7 days (maintenance 5-10 mg/d)	Oral	Peripheral neuropathy can occur with doses >1000 mg daily	21, 22, 29, 37, 41
Pyridoxalphosphate	Active co-factor	Pyridox(am)ine 5′-phosphate oxidase deficiency	40mg/kg/d in 4 divided doses	Oral		29
Riboflavin	Coenzyme	Glutaric aciduria I, mild variants of ETF/ETF-DH and SCAD; congenital lactic acidosis (complex 1 deficiency)	100 mg/d in two to three divided doses	Oral		12, 13, 15, 23

Medication	Mode of action	Disorders	Recommended dose	Route	Remarks	Chapters
Selegiline (*l*-deprenyl)	Monoamine-oxidase-B inhibitor	As adjunct to therapy with 5HT & L-dopa in BH_4 defects	0.1-0.25 mg/d in three to four divided doses	Oral		17
Statins	HMG-CoA reductase inhibitors	Hyperlipidaemias Simvastatin has been used in SLO	► Chapter 32 for discussion regarding use of individual statins	Oral		32, 33
Sodium benzoate	Combines with glycine to form hippuric acid, which has high renal clearance. Removes N_2 and reduces blood ammonia	Hyperammonaemia	250 mg/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
Sodium phenylbutyrate	Converted to phenylacetate, which combines with glutamine to form phenylglutamine which has high renal clearance	Hyperammonaemia	250-650 mg/kg/d; maximum oral dose 20 g/d	Oral or IV		20
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; 7-20 mg/kg/d in PAH def	Oral	May be contraindicated in DHPR deficiency	17
Thiamine	Co-factor	Thiamine responsive variants of MSUD, PDH deficiency & complex 1 def	10-15 mg/d	Oral	Doses of up to 300 mg have been used in CLA; 500-2000 mg/d in thiamine responsive PDH	12,19
Triethylene tetramine (trientine)	Chelating agent	Wilson disease	600 mg/d in divided doses increasing to a maximum of 2.4 g/d if necessary	Oral	May reduce serum iron – iron supplements may be necessary	38
Triheptanoin	Anaplerotic substrate	VLCAD deficiency; PC deficiency	To provide 30% of total calories	Oral		12, 13
Ubiquinone (coenzyme Q10)		inborn errors of CoQ_{10} synthesis	100-300 mg/d	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		36
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

Medication	Mode of action	Disorders	Recommended dose	Route	Remarks	Chapters
Vitamin A	Free radical scavenger	Glutathione synthetase deficiency	100 mg/kg/d	Oral		30
Vitamin C	Co-factor; antioxidant	Hawkinsinuria tyrosinaemia III (4 hydroxyphenylpyruvate dioxygenase deficiency) Transient tyrosinaemia of the newborn Glutathione synthase deficiency	200-1000 mg/d	Oral		18, 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		38

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