Metabolic Disorders

PART IX

Chapter 104

An Approach to Inborn Errors of Metabolism

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Many childhood conditions are caused by single-gene pathogenic variants that encode specific proteins. These pathogenic variants can change the primary protein structure or the amount of protein synthesized. The function of a protein, whether it is an enzyme, receptor, transport vehicle, membrane component, transcriptional coregulator, or structural element, may be compromised or abolished. Hereditary diseases that disrupt normal biochemical processes are termed **inborn errors of metabolism** or **inherited metabolic diseases** (Fig. 104.1).

Most genetic changes are clinically inconsequential and represent *benign* variants. However, pathogenic variants produce diseases that range in severity of presentation and the time of onset. Severe metabolic disorders usually become clinically apparent in the newborn period or shortly thereafter, whereas milder forms may present later in childhood and even in adulthood. With some exceptions, the presenting symptoms of most metabolic conditions lack the specificity to enable a definitive diagnosis without further evaluation. The combination of *low specificity* of presenting symptoms and *low prevalence* of metabolic disorders makes specific diagnosis difficult. Progressive symptoms, the absence of a plausible nongenetic diagnosis after detailed evaluation, history of overlapping symptoms in a patient's relatives, or consanguinity should alert a pediatrician to seek a consultation with a geneticist and prompt metabolic testing early in the evaluation.

Correct diagnosis is often only the beginning of a long medical journey for most families affected by metabolic conditions (see Chapter 95). Although each inherited metabolic disorder is individually rare, improved diagnosis and increasing survival of patients with metabolic conditions virtually ensure that a pediatrician will encounter and provide care to affected patients. Pediatricians can play a critical role in establishing the continuity of care; managing some aspects of treatment; fostering adherence; and delivering routine pediatric interventions such as immunizations, referrals to specialists, and elements of genetic counseling (see Chapter 98.1).

The greater awareness of metabolic conditions, wider availability of biochemical laboratories, global metabolomic analysis, and routine application of exome and genome sequencing dramatically increased the detection rate of the known disorders and contributed to the discovery of new metabolic disorders. Nonetheless, collection and analysis of family history remain critical screening tests that a healthcare provider can use to identify an infant or child at risk for a metabolic disorder. The identification of consanguinity or a particular ethnic background with an unusually high incidence of inborn errors of metabolism can be important to direct further studies. For example, tyrosinemia type 1 is more common among French Canadians of Quebec, maple syrup urine disease is seen with higher frequency in the U.S. Amish population, and Canavan disease is more common in patients of the Ashkenazi Jewish ancestry.

NEWBORN SCREENING

The individual rarity of inborn errors of metabolism, the importance of early diagnosis, and the ensuing genetic counseling ramifications make a strong argument for the universal screening of all newborn infants. **Tandem mass spectrometry** of metabolites and **enzyme assays** form the foundation of first-tier newborn screening. Worldwide, newborn screening programs have begun incorporating reflex second-tier testing using **molecular analysis** of target genes. These methods require only a few drops of blood to be placed on a filter paper and delivered to a central laboratory for assay. Many genetic conditions can be identified by these methods, and the list of disorders continues to grow (Tables 104.1 and 104.2). Pediatricians need to be aware of the general screening procedure and limitations of screening. As a screening method, a positive result may require a repeat newborn screen or confirmatory testing to secure the diagnosis. The time required to return the results varies from country to country and even within states in the same country. Some metabolic conditions can be severe enough to cause clinical manifestations before the results of the newborn screening become available. Conversely, diagnostic metabolites in milder forms of screened disorders may not reach a set threshold to trigger secondary studies, thus leading to negative newborn screen results and delayed diagnosis. *Therefore negative newborn screening in a patient with symptoms suggestive of a metabolic disorder warrants a referral to a genetics specialist for further evaluation.*

Universal newborn screening may also identify mild forms of inherited metabolic conditions, some of which may never cause clinical manifestations in the lifetime of the individual. For example, short-chain acyl-CoA dehydrogenase deficiency has been identified with unexpectedly high frequency in screening programs using tandem mass spectrometry, but most of these children have remained asymptomatic. This highlights the need for an ongoing evaluation of metabolite cutoff values and approaches to confirmatory testing to maximize the diagnostic yield and minimize potential psychosocial and economic implications of such findings. Premature infants represent a special population in whom the incidence of false-positive or false-negative test results can be especially high.

With the advent of genetic therapy for spinal muscular atrophy (SMA) and enzyme replacement therapy for some lysosomal storage diseases (e.g., Pompe disease, Fabry disease, Gaucher disease, and mucopolysaccharidosis type 1), most state newborn screening programs include screening for SMA and lysosomal storage disorders.

CLINICAL MANIFESTATIONS OF GENETIC METABOLIC DISEASES

Physicians and other healthcare providers who care for children should familiarize themselves with early manifestations of inborn errors of metabolism, because (1) severe forms of some of these conditions may cause symptoms before the results of screening studies become available and (2) the current screening methods, although quite extensive, identify a small number of all inherited metabolic conditions. In the newborn period, the clinical findings are usually nonspecific and similar to those seen in infants with sepsis. An inborn error of metabolism should be considered in the differential diagnosis of a severely ill newborn infant, and special studies should be undertaken if the index of suspicion is high (see Fig. 104.1).

Signs and symptoms such as lethargy, hypotonia, hypothermia, convulsions (Table 104.3), poor feeding, and vomiting may develop as early as a few hours after birth. Occasionally, vomiting may be severe enough to suggest the diagnosis of pyloric stenosis, which is usually not present, although it may occur simultaneously in some infants.

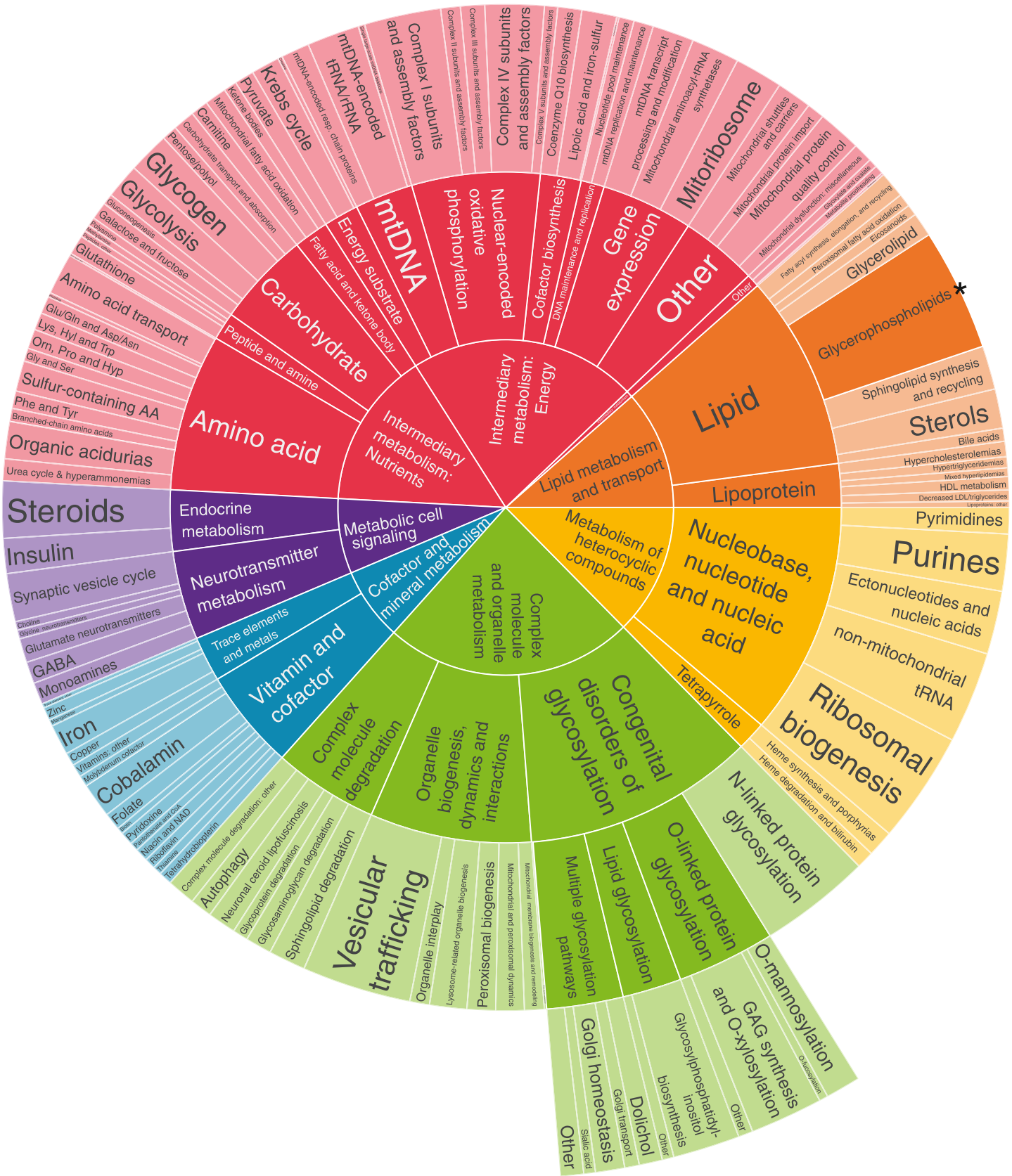

Fig. 104.1 Sunburst chart depicting the hierarchical nature of the International Classification of Inherited Metabolic Disorders. The size of each section of the chart is directly proportional to the number of disorders in that group. *Including phosphatidylinositol (with lesser numbers for ether lipids and lesser still for phosphatidylcholine, phosphatidylserine, and phosphatidylethanolamine) (*From Ferreira CR, Rahman S, Keller M, et al. An international classification of inherited metabolic disorders [ICIMD]. J Inherit Metab Dis. 2021;44:164–177, Fig. 1.*)

Table 104.1 Core Conditions on the Recommended Uniform Screening Panel by the Advisory Committee on Heritable Disorders in Newborns and Children of the U.S. Health Resources & Services Administration*

DISORDERS OF ORGANIC ACID METABOLISM
Propionic acidemia
Methylmalonic acidemia (methylmalonyl-CoA mutase)
Methylmalonic acidemia (cobalamin disorders)
Isovaleric acidemia
3-Methylcrotonyl-CoA carboxylase deficiency
3-Hydroxy-3-methyglutaric aciduria
Holocarboxylase synthase deficiency
β-Ketothiolase deficiency
Glutaric acidemia type I

DISORDERS OF FATTY ACID METABOLISM
Medium-chain acyl-CoA dehydrogenase deficiency
Very-long-chain acyl-CoA dehydrogenase deficiency
Long-chain 3-hydroxy-acyl-CoA dehydrogenase deficiency
Trifunctional protein deficiency
Systemic primary carnitine deficiency

DISORDERS OF AMINO ACID METABOLISM
Classic phenylketonuria
Maple syrup urine disease
Homocystinuria
Citrullinemia type 1
Argininosuccinic acidemia
Tyrosinemia type I

HEMOGLOBINOPATHIES
Sickle cell anemia (hemoglobin SS disease)
Hemoglobin S/β-thalassemia
Hemoglobin S/C disease

ENDOCRINE DISORDERS
Primary congenital hypothyroidism
Congenital adrenal hyperplasia

OTHER DISORDERS
Classic galactosemia
Biotinidase deficiency
Glycogen storage disease type II (Pompe disease)
Mucopolysaccharidosis type 1
X-linked adrenoleukodystrophy
Cystic fibrosis
Hearing loss
Severe combined immunodeficiency (SCID)
Critical congenital heart disease
Spinal muscular atrophy (SMA) due to homozygous deletion of exon 7 in *SMN1*

*Adopted from https://www.hrsa.gov/advisory-committees/heritable-disorders/rusp/index.html, last revised in February 2020.

Table 104.2 Secondary Conditions on the Recommended Uniform Screening Panel by the Advisory Committee on Heritable Disorders in Newborns and Children of the U.S. Health Resources & Services Administration

ORGANIC ACID METABOLISM DISORDERS
Methylmalonic acidemia with homocystinuria
Malonic acidemia
2-Methyl-3-hydroxybutyric aciduria
Isobutyryl-CoA dehydrogenase deficiency
2-Methylbutyryl-CoA dehydrogenase deficiency
3-Methylglutaconic aciduria

FATTY ACID OXIDATION DISORDERS
Short-chain acyl-CoA dehydrogenase deficiency
Glutaric acidemia type 2
Medium-/short-chain 3-hydroxy-acyl-CoA dehydrogenase deficiency
Medium-chain ketoacyl-CoA thiolase deficiency
Carnitine palmitoyltransferase IA deficiency
Carnitine palmitoyltransferase II deficiency
Carnitine-acylcarnitine translocase deficiency
2,4-Dienoyl-CoA reductase deficiency

AMINO ACID METABOLISM DISORDERS
Hyperphenylalaninemia, benign (not classic phenylketonuria)
Tyrosinemia type II
Tyrosinemia type III
Defects of biopterin cofactor biosynthesis
Defects of biopterin cofactor regeneration
Argininemia
Hypermethioninemia
Citrullinemia type II (citrin deficiency)

HEMOGLOBINOPATHIES
Hemoglobin variants (including hemoglobin E)

OTHERS
Galactose epimerase deficiency
Galactokinase deficiency
T-cell–related lymphocyte deficiencies

Table 104.3 Select Inborn Errors of Metabolism Associated with Neurologic and Laboratory Manifestations in Neonates

DETERIORATION IN CONSCIOUSNESS
Metabolic Acidosis
Organic acidemias
Disorders of pyruvate metabolism
Fatty acid oxidation defects
Fructose-1,6-bisphosphatase deficiency
Glycogen storage diseases
Mitochondrial respiratory chain defects
Disorders of ketone metabolism

HYPOGLYCEMIA*
Fatty acid oxidation defects
Disorders of gluconeogenesis
Disorders of fructose and galactose metabolism
Glycogen storage diseases
Disorders of ketogenesis
Organic acidemias
Hyperinsulinemic hypoglycemias
Mitochondrial respiratory chain defects
Neonatal intrahepatic cholestasis caused by citrin deficiency
Disorders of pyruvate metabolism
Carbonic anhydrase VA deficiency

Continued

Table 104.3 Select Inborn Errors of Metabolism Associated with Neurologic and Laboratory Manifestations in Neonates—cont'd

HYPERAMMONEMIA**
Urea cycle disorders
Organic acidemias
Fatty acid oxidation disorders
Disorders of pyruvate metabolism
GLUD1-related hyperinsulinemic hypoglycemia
Carbonic anhydrase VA deficiency

SEIZURES AND HYPOTONIA
Antiquitin deficiency (pyridoxine-dependent epilepsy)
Pyridoxamine 5′-phosphate oxidase (PNPO) deficiency (pyridoxal phosphate-responsive epilepsy)
Folate metabolism disorders
Multiple carboxylase deficiency (holocarboxylase synthetase deficiency and biotinidase deficiency)
Urea cycle disorders
Organic acidemias
Fatty acid oxidation disorders
Disorders of creatine biosynthesis and transport
Disorders of neurotransmitter metabolism
Molybdenum cofactor deficiency and sulfite oxidase deficiency
Serine deficiency disorders
Glycine encephalopathy
Asparagine synthetase deficiency
Mitochondrial respiratory chain defects
Zellweger spectrum disorders
Congenital disorders of glycosylation
Purine and pyrimidine metabolism defects

NEONATAL APNEA
Glycine encephalopathy
Asparagine synthetase deficiency
Urea cycle disorders
Organic acidemias
Disorders of pyruvate metabolism
Fatty acid oxidation defects
Mitochondrial respiratory chain defects

*Refer to Table 104.4 for more details on the metabolic disorders associated with neonatal hypoglycemia.
**Refer to Table 104.5 for more details on the differential diagnosis of neonatal and infantile hyperammonemia.
Modified from El-Hattab AW. Inborn errors of metabolism. *Clin Perinatol.* 2015;42:413–439, Box 1.

Table 104.4 Select Inborn Errors of Metabolism Associated with Neonatal Hypoglycemia

CATEGORY OF DISORDERS	DISORDERS
Fatty acid oxidation disorders	Carnitine-acylcarnitine translocase deficiency Carnitine palmitoyltransferase Ia deficiency Carnitine palmitoyltransferase II deficiency Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency/trifunctional protein deficiency Medium-chain acyl-CoA dehydrogenase deficiency Very-long-chain acyl-CoA dehydrogenase deficiency Multiple acyl-CoA dehydrogenase deficiency
Disorders of gluconeogenesis	Fructose-1,6-diphosphatase deficiency Phosphoenolpyruvate carboxykinase deficiency
Disorders of fructose and galactose metabolism	Hereditary fructose intolerance Classic galactosemia
Glycogen storage diseases (GSD)	GSD type Ia (glucose-6-phosphatase deficiency) GSD type Ib (impaired glucose-6-phosphate exchanger) GSD type III (glycogen debrancher enzyme deficiency) GSD type VI (liver glycogen phosphorylase deficiency) GSD type IX (phosphorylase kinase deficiencies)
Disorders of ketone metabolism	3-Hydroxy-3-methylglutaryl-CoA lyase deficiency Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase deficiency Succinyl-CoA:3-oxoacid-CoA transferase (SCOT) deficiency β-Ketothiolase deficiency
Disorders of amino acid metabolism	Maple syrup urine disease
Organic acidemias	Propionic acidemia Methylmalonic acidemia Isovaleric acidemia Multiple carboxylase deficiency (holocarboxylase synthetase deficiency and biotinidase deficiency)
Hyperinsulinemic hypoglycemia	*HADH*-related disorder (3-alpha-hydroxyacyl-CoA dehydrogenase deficiency) *GLUD1*-related disorder (hyperammonemia-hyperinsulinism syndrome [HIHA])
Other	Mitochondrial respiratory chain defects Neonatal intrahepatic cholestasis caused by citrin deficiency Pyruvate carboxylase deficiency Carbonic anhydrase VA deficiency

Modified from Zinn AB. Inborn errors of metabolism. In: Martin RJ, Fanaroff AA, Walsh MC, eds. *Fanaroff & Martin's Neonatal-Perinatal Medicine: Diseases of the Fetus and Infant,* 10th ed. Philadelphia: Elsevier; 2015: Table 99.17, p. 1605.

Lethargy, poor feeding, seizures, and coma may also be seen in infants with hypoglycemia (Table 104.4) (see Chapters 113 and 147), hypocalcemia (Chapters 69 and 611), and hyperammonemia (Table 104.5) (Chapter 105). Measurements of blood concentrations of glucose and calcium and prompt response to intravenous injection of glucose or calcium help guide the diagnostic decisions.

Every organ can be affected by metabolic disorders. However, *physical examination* usually reveals nonspecific findings; most signs are related to the central nervous system such as lethargy, coma, seizures, hyperventilation, or opisthotonus. Hepatomegaly is a common finding in a variety of inborn errors of metabolism (Table 104.6). Cardiomyopathy (Table 104.7), dysmorphic features (Table 104.8),

Table 104.5 Differential Diagnosis of Hyperammonemia

INBORN ERRORS OF METABOLISM
Urea Cycle Enzyme Defects
N-acetylglutamate synthase (NAGS) deficiency
Carbamoyl phosphate synthetase one (CPS1) deficiency
Ornithine transcarbamylase (OTC) deficiency
Argininosuccinate synthetase (ASS) deficiency (citrullinemia type 1)
Argininosuccinate lyase (ASL) deficiency (argininosuccinic aciduria)
Arginase 1 deficiency
Transport and Synthesis Defects of Urea Cycle Intermediates
Hyperornithinemia-hyperammonemia-homocitrullinemia (HHH syndrome)
Citrullinemia type 2 caused by citrin deficiency
Lysinuric protein intolerance
Ornithine aminotransferase deficiency
Carbonic anhydrase VA deficiency
Organic Acidemias
Propionic acidemia
MMUT-related methylmalonic acidemia and cobalamin metabolism disorders
Isovaleric acidemia
Fatty Acid Oxidation Disorders
Long-chain fatty acid oxidation defects
Systemic primary carnitine deficiency
Other
Pyruvate carboxylase deficiency
GLUD1-related hyperinsulinemic hypoglycemia
Neonatal iron overload disorders (e.g., hereditary hemochromatoses)

ACQUIRED DISORDERS
Transient Hyperammonemia of the Newborn
Diseases of the Liver and Biliary Tract
Liver failure
Biliary atresia
Severe Systemic Neonatal Illness
Neonates sepsis
Heart failure
Medications
Valproic acid
Cyclophosphamide
5-Pentanoic acid
Asparaginase
Other
Reye syndrome

ANATOMIC VARIANTS
Vascular bypass of the liver (e.g., a portosystemic anastomosis)

TECHNICAL
Inappropriate sample collection (e.g., capillary blood or prolonged placement of a tourniquet)
Sample not immediately analyzed

Modified from El-Hattab AW. Inborn errors of metabolism. *Clin Perinatol*. 2015;42:413–439, Box 8.

and fetal hydrops (Table 104.9) are additional potential manifestations of a metabolic disorder (Table 104.10). Occasionally, a peculiar odor may offer an invaluable clue leading to the right diagnosis (Table 104.11).

In an increasing number of patients, a metabolic condition may be recognized months or years after birth. This is more typical in patients carrying milder autosomal recessive pathogenic variants, in mitochondrial disorders, in females affected by X-linked recessive conditions, and specific metabolic conditions that usually present later in life. There may be an episodic or intermittent pattern, with episodes of acute clinical manifestations separated by periods of seemingly disease-free states. The episodes are usually triggered by stress or nonspecific catabolic stress such as an infection, overfeeding, prolonged fasting, or physical exertion. Thus an inborn error of metabolism should be considered in any child with one or more of the following unexplained clinical manifestations: failure to thrive; coarse facial features and reduced range of motion in the joints; developmental delay; intellectual disability; developmental regression; motor deficits or adventitious movements (e.g., dystonia, choreoathetosis, ataxia); seizures; catatonia; myopathy; intermittent episodes of unexplained vomiting, acidosis, mental deterioration, psychosis, or coma; hepatomegaly; renal stones; renal dysfunction, especially Fanconi syndrome or renal tubular acidosis; cardiomyopathy; persistent leukopenia; megaloblastic anemia; and unusual odor (particularly during an acute illness) (Table 104.12).

Diagnosis usually requires a variety of specific laboratory studies. Plasma amino acid analysis, total plasma homocysteine, plasma acylcarnitine profile, total and free carnitine levels, and urine organic acid assay, although not exhaustive in their diagnostic scope, are useful as initial screening tests to evaluate for a suspected inborn error of metabolism. Measurements of plasma ammonia, glucose, lactate, bicarbonate, and pH are readily available in hospitals and very helpful *initially* in differentiating major causes of genetic metabolic disorders (Table 104.13 and Fig. 104.2). Elevation of blood ammonia is usually caused by defects of urea cycle enzymes, organic acidemias, and disorders of fatty acid oxidation. Infants with elevated blood ammonia levels from urea cycle defects tend to have normal serum pH and bicarbonate values; without measurement of blood ammonia, they may remain undiagnosed and succumb to their disease. In organic acidemias, elevated plasma ammonia is accompanied by severe acidosis caused by accumulation of organic acids, ketone bodies, and lactate in body fluids.

When blood ammonia, pH, and bicarbonate values are normal, other aminoacidopathies (e.g., hyperglycinemia) or galactosemia should be considered. Galactosemic infants may also manifest cataracts, hepatomegaly, ascites, and jaundice.

Currently, more targeted assays, such as those used with newborn screening, have the highest sensitivity in the diagnosis or monitoring of specific disorders in question. However, some success has been shown in the context of undiagnosed individuals where metabolomic (assays for entire small molecule metabolites) data can be correlated with genomic information to provide supporting evidence for a pathogenic variant in a suspected causal gene.

TREATMENT

Most patients with genetic disorders of metabolism respond to one or more of the following treatments:

1. Special diets play an important role in the treatment of affected children. Dietary changes should be tailored to the pathophysiology of the condition and vary greatly among disorders.
2. Hemodialysis for expeditious removal of accumulated noxious compounds. This is a very effective modality for treatment of the acute phase of the condition.
3. Catabolic states in patients at risk for metabolic crisis can be treated with fluids containing dextrose and electrolytes.
4. Administration of the deficient metabolite.
5. Administration of the cofactor or coenzyme to maximize the residual enzyme activity.
6. Activation of alternative pathways to reduce the noxious compounds accumulated because of the genetic abnormality.
7. Administration of the deficient enzyme.
8. Bone marrow transplantation.
9. Liver and kidney transplantation.

Table 104.6 Select Metabolic Disorders Associated with Hepatic Dysfunction

CATEGORY OF DISORDERS	DISORDERS
Disorders of amino acid metabolism	Tyrosinemia type I Citrullinemia type II caused by citrin deficiency Disorders of methionine metabolism Urea cycle disorders
Biliary tract disorders and disorder of bile acid synthesis	See Chapter 383
Disorders of fructose and galactose metabolism	Hereditary fructose intolerance Classic galactosemia Epimerase deficiency galactosemia
Congenital disorders of glycosylation	Multiple types
Fatty acid oxidation disorders	Carnitine-acylcarnitine translocase deficiency Carnitine palmitoyltransferase Ia deficiency Carnitine palmitoyltransferase II deficiency Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency/trifunctional protein deficiency Very-long-chain acyl-CoA dehydrogenase deficiency Multiple acyl-CoA dehydrogenase deficiency
Glycogen storage disorders (GSD)	GSD type 1a (deficiency of glucose-6-phosphatase catalytic activity) GSD type 1b (a defect in glucose-6-phosphate exchanger encoded by *SLC37A4*) GSD type III (glycogen debrancher enzyme deficiency) GSD type IV (glycogen branching enzyme deficiency) GSD type VI (liver glycogen phosphorylase deficiency)
Peroxisomal disorders	Zellweger spectrum disorders Disorders of peroxisomal β-oxidation
Mitochondrial respiratory chain (RC) defects	Mitochondrial DNA (mtDNA) or nuclear DNA (nDNA) defects: Specific single-nucleotide pathogenic variants in mtDNA Large-scale mtDNA re-arrangements (Pearson syndrome) Disorders of mitochondrial translation (e.g., $tRNA^{Glu}$) Disorder of protein synthesis of RC complexes Disorders affected the assembly or stabilization of RC complexes (e.g., *BCS1L*) Disorders of cofactor biosynthesis (e.g., coenzyme Q10) Disorders of mitochondrial transport and dynamics mtDNA depletion syndromes (e.g., *DGUOK, MPV17, POLG, SUCLG1*)
Lysosomal storage disorders	Niemann-Pick disease type C
Other	α_1-Antitrypsin deficiency

Modified from Zinn AB. Inborn errors of metabolism. In: Martin RJ, Fanaroff AA, Walsh MC, eds. *Fanaroff & Martin's Neonatal-Perinatal Medicine: Diseases of the Fetus and Infant,* 10th ed. Philadelphia: Elsevier; 2015: Table 99.5, p. 1579.

Table 104.7 Select Metabolic Disorders Associated with Cardiomyopathy

CATEGORY OF DISORDERS	DISORDERS
Organic acidemias	Propionic acidemia Cobalamin C deficiency 3-methylglutaconic acidurias (e.g., Barth syndrome and DCMA syndrome)
Lysosomal storage disorders	Sphingolipidoses (e.g., Fabry disease) Oligosaccharidoses and mucolipidoses (e.g., I-cell disease) Mucopolysaccharidoses
Glycogen storage disorders (GSD)	GSD type II (Pompe disease) GSD type III (glycogen debrancher enzyme deficiency) *PRKAG2*-related disorders (includes lethal congenital glycogen storage disease of heart)
Congenital disorders of glycosylation	Multiple types
Fatty acid oxidation disorders	Carnitine-acylcarnitine translocase deficiency Carnitine palmitoyltransferase II deficiency Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency/trifunctional protein deficiency *ACAD9*-related disorder (mitochondrial acyl-CoA dehydrogenase deficiency) Multiple acyl-CoA dehydrogenase deficiency (includes glutaric aciduria type 2) Very-long-chain acyl-CoA dehydrogenase deficiency Systemic primary carnitine deficiency
Mitochondrial respiratory chain (RC) defects	Mitochondrial DNA (mtDNA) or nuclear DNA (nDNA) defects: Specific single-nucleotide pathogenic variants in mtDNA Large-scale mtDNA deletions Disorders of mitochondrial translation (e.g., $tRNA^{Leu}$) Disorders of protein synthesis of RC complexes (e.g., *MT-ATP6, MT-ATP8, NDUFS2, NDUFV2, SDHA, SCO2, COX10, COX15*) Disorders affecting the assembly or stabilization of RC complexes (e.g., *TMEM70*) Disorders of cofactor biosynthesis (e.g., coenzyme Q10) Disorders of mitochondrial transport and dynamics (e.g., *SLC25A3*) mtDNA depletion syndromes (e.g., *SUCLG1*)
Other	Danon disease

DCMA, dilated cardiomyopathy with ataxia.
Modified from Zinn AB. Inborn errors of metabolism. In: Martin RJ, Fanaroff AA, Walsh MC, eds. *Fanaroff & Martin's Neonatal-Perinatal Medicine: Diseases of the Fetus and Infant,* 10th ed. Philadelphia: Elsevier; 2015: Table 99.4, p. 1576.

Table 104.8 Select Inborn Errors of Metabolism Associated with Dysmorphic Features

CATEGORY OF DISORDERS	DISORDERS
Congenital disorders of glycosylation	N-Glycosylation disorders (e.g., PMM2-CDG and ALG3-CDG) O-Glycosylation disorders (e.g., Walker-Warburg syndrome)
Disorders of cholesterol biosynthesis	Smith-Lemli-Opitz syndrome Desmosterolosis Lathosterolosis *EBP*-related disorder (includes Conradi-Hunermann syndrome)
Lysosomal storage disorders	Sphingolipidoses Oligosaccharidoses and mucolipidoses Mucopolysaccharidoses
Organic acidurias	Multiple acyl-CoA dehydrogenase deficiency (includes glutaric aciduria type 2) Mevalonic aciduria*
Peroxisomal disorders	Zellweger spectrum disorders Disorders of peroxisomal β-oxidation
Other	Pyruvate dehydrogenase complex deficiency

*Mevalonic aciduria has been classified as an organic acidemia based on the method used for its diagnosis, but it can also be classified as a peroxisomal single-enzyme disorder or as a defect in cholesterol biosynthesis because of its intracellular location or function.

Modified from Zinn AB. Inborn errors of metabolism. In: Martin RJ, Fanaroff AA, Walsh MC, eds. *Fanaroff & Martin's Neonatal-Perinatal Medicine: Diseases of the Fetus and Infant,* 10th ed. Philadelphia: Elsevier; 2015: Table 99.8, p. 1583.

Table 104.9 Select Inborn Errors of Metabolism Associated with Hydrops Fetalis

LYSOSOMAL STORAGE DISORDERS	ZELLWEGER SPECTRUM DISORDERS
Mucopolysaccharidoses types I, IVA, and VII	Glycogen storage disease type IV
Sphingolipidoses (e.g., Gaucher disease, Farber disease, Niemann-Pick disease A, GM_1 gangliosidosis, multiple sulfatase deficiency)	Congenital disorders of glycosylation
Lipid storage diseases (Wolman and Niemann-Pick disease C)	Mitochondrial respiratory chain defects
Oligosaccharidases (e.g., sialidosis type I)	Transaldolase deficiency
Mucolipidoses (e.g., I-cell disease)	

Modified and adapted from El-Hattab AW. Inborn errors of metabolism. *Clin Perinatol.* 2015;42:413–439, Box 6.

Table 104.10 Physical Examination Findings Associated with Inborn Errors of Metabolism (Select Examples)

FINDINGS	DISORDERS
Hepatomegaly	Disorders of fructose and galactose metabolism (e.g., classic galactosemia and hereditary fructose intolerance) Glycogen storage diseases Disorders of gluconeogenesis Disorders of fatty acid oxidation and transport Mitochondrial respiratory chain defects Tyrosinemia type 1 Urea cycle disorders Zellweger spectrum disorders Niemann-Pick disease type C Congenital disorders of glycosylation
Hepatosplenomegaly	Mucopolysaccharidoses Niemann-Pick disease types A, B, and C Sphingolipidoses (e.g., GM_1 gangliosidosis or Gaucher disease) Wolman disease Farber disease (acid ceramidase deficiency)
Macrocephaly	Glutaric acidemia type 1 Canavan disease
Microcephaly	Mitochondrial respiratory chain defects Disorders of intracellular cobalamin metabolism (e.g., *cblC* deficiency) Cholesterol metabolism disorders (e.g., Smith-Lemli-Opitz syndrome) Serine synthesis disorders
Coarse facial features	Mucopolysaccharidoses Oligosaccharidoses and mucolipidoses (e.g., α-mannosidosis) Sphingolipidoses (e.g., GM_1 gangliosidosis) Galactosialidosis
Macroglossia	Glycogen storage disease type II (Pompe disease) Mucopolysaccharidoses Oligosaccharidoses and mucolipidoses Sphingolipidoses Galactosialidosis
Dystonia or extrapyramidal signs	Gaucher disease type 2 Glutaric acidemia type 1 Methylmalonic acidemia Propionic acidemia Krabbe disease Crigler-Najjar syndrome Disorders of neurotransmitter metabolism Pyruvate dehydrogenase complex deficiency
Macular "cherry-red spot"	GM_1 gangliosidosis Tay-Sachs disease (GM_2 gangliosidosis) Farber disease (acid ceramidase deficiency) Galactosialidosis Niemann-Pick disease type A Sialidosis Multiple sulfatase deficiency
"Bull eye" maculopathy	*cblC* and *cblD* deficiency (combined methylmalonic acidemia and homocystinuria, type C)
Retinitis pigmentosa	Mitochondrial respiratory chain defects Peroxisomal disorders Abetalipoproteinemia
Optic nerve atrophy or hypoplasia	Pyruvate dehydrogenase complex deficiency Mitochondrial respiratory chain defects Peroxisomal disorders Propionic acidemia *MMUT*-related methylmalonic acidemia and cobalamin metabolism disorders

Continued

Table 104.10 Physical Examination Findings Associated with Inborn Errors of Metabolism (Select Examples)—cont'd

FINDINGS	DISORDERS
Corneal clouding or opacities	Mucolipidoses Mucopolysaccharidoses Steroid sulfatase deficiency Tyrosinemia type II Cystinosis
Cataracts	Disorders of galactose metabolism (e.g., classic galactosemia) Congenital disorders of glycosylation Mitochondrial respiratory chain (RC) defects Peroxisomal disorders Lowe oculocerebrorenal syndrome
Dislocated lens	Cystathionine β-synthase deficiency Molybdenum cofactor deficiency and sulfite oxidase deficiency
Skeletal dysplasias and dysostosis multiplex	Oligosaccharidoses and mucolipidoses Mucopolysaccharidoses Sphingolipidoses Galactosialidosis Peroxisomal disorders Disorders of cholesterol biosynthesis Congenital disorders of glycosylation
Thick skin	Oligosaccharidoses and mucolipidoses Mucopolysaccharidoses Sphingolipidoses
Desquamating, eczematous, or vesiculobullous skin lesions	Acrodermatitis enteropathica Essential amino acid deficiencies in organic acidemias Hartnup disorder Multiple carboxylase deficiency (holocarboxylase synthetase deficiency and biotinidase deficiency) Porphyrias
Ichthyosis	Gaucher disease type 2 Steroid sulfatase deficiency Refsum disease *ELOVL4*-related disorder Serine deficiency disorders
Alopecia	Multiple carboxylase deficiency (holocarboxylase synthetase deficiency and biotinidase deficiency)
Steely or kinky hair	Menkes disease
Trichorrhexis nodosa	Argininosuccinic aciduria (ASL deficiency)
Megaloblastic anemia	Cobalamin metabolism disorders Folate metabolism disorders Mevalonic aciduria Orotic aciduria Pearson syndrome
Leukopenia	Folate metabolism disorders Organic acidemias Pearson syndrome
Persistent diarrhea	Glucose-galactose malabsorption Congenital lactase deficiency Congenital chloride diarrhea Sucrase-isomaltase deficiency Acrodermatitis enteropathica Abetalipoproteinemia Congenital folate malabsorption Wolman disease Lysinuric protein intolerance Classic galactosemia

Modified from Cederbaum S. Introduction to metabolic and biochemical genetic diseases. In: Gleason CA, Juul SE, eds. *Avery's Diseases of the Newborn*, 10th ed. Philadelphia: Elsevier; 2018: Table 21.1, p. 227.

Table 104.11 Inborn Errors of Amino Acid Metabolism Associated with Peculiar Odor

INBORN ERROR OF METABOLISM	URINE ODOR
Isovaleric acidemia Glutaric acidemia (type II)	"Sweaty feet," acrid
Maple syrup urine disease	Maple syrup, burnt sugar
Multiple carboxylase deficiency 3-Methylcrotonyl-CoA carboxylase deficiency 3-Hydroxy-3-methylglutaric aciduria	Cat urine
Phenylketonuria	Mousey or musty
Trimethylaminuria Dimethylglycine dehydrogenase deficiency	Rotten fish
Tyrosinemia type 1	Boiled cabbage, rancid butter
Hypermethioninemia	Boiled cabbage
Cystinuria Tyrosinemia type I	Sulfur
Hawkinsinuria	"Swimming pool"
Oasthouse urine disease	Hopslike

In select inborn errors of metabolism, organ transplantation modalities may offer the best treatment modality to stabilize a metabolic patient and improve quality of life. To date, replacement of the affected gene with a normal copy using gene therapy has been successful in only a few diseases.

Treatment of genetic disorders of metabolism is complex and requires medical and technical expertise. Effective treatment is best achieved by a team of specialists—metabolic genetics specialist, nutritionist, neurologist, and psychologist—in a major medical center. The therapeutic regimen often needs to be tailored to the individual patient because of large phenotypic variations in the severity of the disease, even within a single family. Providing genetic counseling, education, and ongoing social services support for the family is the key to successful long-term therapy. Even in patients with poor prognoses, every effort should be made to establish correct diagnoses premortem.

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Table 104.12 Clinical Findings That Should Prompt a Metabolic Workup

Family history	Sibling(s) who died from unexplained causes or exhibit overlapping symptoms Ethnic groups with a high prevalence of metabolic disorders Consanguinity
Perinatal history	Intrauterine growth restriction, sepsis-like presentation in the neonatal period, nonimmune fetal hydrops
Growth	Postnatal failure to thrive, microcephaly, macrocephaly, short stature
Central and peripheral nervous systems	Progressive encephalopathy, lethargy, coma, intractable seizures, developmental delay, developmental regression, intellectual disability, autism spectrum disorder, hypotonia, spasticity, dystonia, strokes, ataxia, psychosis, intracranial calcifications, white matter disease, peripheral neuropathy
Respiratory system	Hyperventilation, apnea
Cardiovascular system	Cardiac failure with or without cardiomyopathy, arrhythmia
Musculoskeletal system	Rhabdomyolysis, myopathy Osteopenia, early-onset osteoporosis, skeletal dysplasia, epiphyseal abnormalities, bone crises
Eye	Retinitis pigmentosa, macular dystrophy, cataracts, corneal opacities, nystagmus, cherry-red spot
Hearing	Sensorineural hearing loss
Gastrointestinal system	Hepatomegaly, hepatic adenoma, splenomegaly, liver failure, Reye syndrome, cholestasis, cirrhosis, chronic diarrhea, vomiting, acute pancreatitis
Kidney	Renal dysfunction, renal Fanconi syndrome, renal stones
Hematologic system	Anemia, leukopenia, thrombocytopenia, pancytopenia, hemolytic-uremic syndrome
Skin	Hair abnormality, alopecia, lipodystrophy, recalcitrant eczema

Table 104.13 Laboratory Findings That Should Prompt a Metabolic Workup

Hyperammonemia	Hypoglycemia
Metabolic acidosis	Liver dysfunction
Lactic acidosis	Pancytopenia
Ketosis	

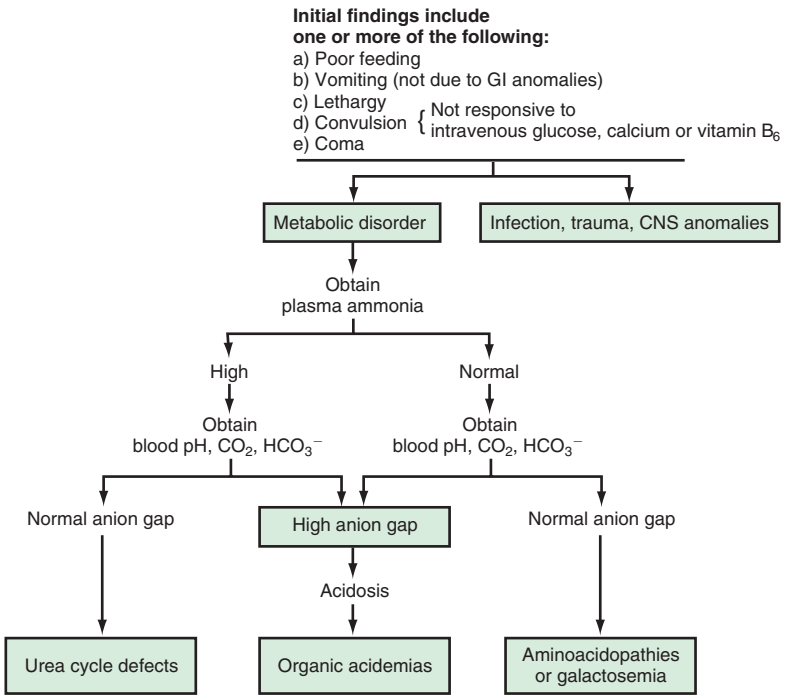

Fig. 104.2 Algorithm showing the initial clinical approach to a full-term newborn infant with a suspected genetic metabolic disorder. This schema is a guide to elucidate some of the metabolic disorders in newborn infants. Although some exceptions to this schema exist, it is appropriate for most cases affected by disorders or intermediate metabolism. CNS, Central nervous system; GI, gastrointestinal; HCO_3^-, bicarbonate.

Chapter 105

Defects in Metabolism of Amino Acids

105.1 Phenylalanine

Oleg A. Shchelochkov and Charles P. Venditti

Phenylalanine is an essential amino acid. Dietary phenylalanine that is not used for protein synthesis is normally degraded by way of the tyrosine pathway (Fig. 105.1). Deficiency of the enzyme **phenylalanine hydroxylase (PAH)** or one of its cofactors **tetrahydrobiopterin (BH_4)** causes accumulation of phenylalanine and its metabolites in body fluids and in the brain.

Elevations of phenylalanine in the plasma reflect the degree of enzyme deficiency, presenting as a spectrum of biochemical and clinical findings. In patients with **severe PAH deficiency** (also referred to as *classical phenylketonuria*), plasma phenylalanine levels on an unrestricted diet usually exceed 20 mg/dL (>1,200 μmol/L). Patients with milder PAH pathogenic variants have plasma phenylalanine levels between 10 mg/dL (600 μmol/L) and 20 mg/dL (1,200 μmol/L). Levels between 2 and 10 mg/dL (120 and 600 μmol/L) on an unrestricted diet are observed in patients with **mild hyperphenylalaninemia**. In affected infants with plasma concentrations >20 mg/dL, excess phenylalanine is metabolized to phenylketones (phenylpyruvate and phenylacetate; see Fig. 105.1) that are excreted in the urine, giving rise to the term *phenylketonuria* (PKU). These metabolites have no known role in the mechanisms of central nervous system (CNS) damage in PKU patients, but their presence in body fluids can signify the severity of the condition. The **brain** is most vulnerable to the damage incurred by PKU, but the exact mechanism of injury remains elusive. Both toxic elevations of phenylalanine and insufficient tyrosine may play roles. Phenylalanine hydroxylase converts phenylalanine to **tyrosine**, which is necessary for the production of neurotransmitters such as epinephrine, norepinephrine, and dopamine (Fig. 105.2). If the degree of enzymatic block is severe, tyrosine becomes an essential amino acid and may be deficient if its intake is not adequate. On the other hand, observations that lower concentrations of phenylalanine in plasma and brain tissue are associated with improved neurobehavioral outcomes support the view that toxic levels of phenylalanine are key to PKU pathogenesis. High blood levels of phenylalanine can saturate the transport system across the blood-brain barrier (BBB) and inhibit the cerebral uptake of other large neutral amino acids such as the branched-chain amino acids tyrosine and tryptophan, impairing brain protein synthesis.

SEVERE PHENYLALANINE HYDROXYLASE DEFICIENCY (CLASSIC PHENYLKETONURIA)

Elevations of plasma phenylalanine >20 mg/dL (>1,200 μmol/L), if untreated, invariably result in the development of signs and symptoms of classic PKU, except in uncommon and unpredictable cases.

Clinical Manifestations

The affected infant appears normal at birth. Profound intellectual disability gradually develops if the infant remains untreated. Cognitive delay may not be evident for the first few months. In untreated patients, 50–70% will have an IQ below 35, and 88–90% will have an IQ below 65. Less than 5% of untreated patients attain IQ scores in the average range. Vomiting, sometimes severe enough to be misdiagnosed as pyloric stenosis, may be an early symptom. Older untreated children become hyperactive and show autistic behaviors, including stereotypic hand movements and rhythmic rocking.

Untreated and undertreated infants are lighter in their complexion than unaffected siblings. Some may have a seborrheic or eczematoid rash, which is usually mild and disappears with age. Affected children have an odor of phenylacetic acid, which has been described as musty or "mousey." Neurologic signs include seizures (approximately 25%), spasticity, hyperreflexia, tremors, and athetosis; >50% have electroencephalographic (EEG) abnormalities. Microcephaly, prominent maxillae with widely spaced teeth, enamel hypoplasia, and growth retardation are other common findings in untreated children. Low bone mineral density and osteopenia have been reported in affected individuals of all ages. Although inadequate intake of natural proteins seems to be the major culprit, the exact pathogenesis of this sequela remains unclear.

Long-term care of patients with PKU is best achieved by a team of experienced professionals (metabolic specialist, nutritionist, and psychologist), typically in a regional treatment center. The clinical manifestations of classical PKU are rarely seen in countries where neonatal screening programs for the detection of PKU are in effect.

Non-PKU Hyperphenylalaninemia

Implementation of universal screening for PKU led to identification of a group of infants in whom initial plasma concentrations of phenylalanine are above normal (i.e., >2 mg/dL, or 120 μmol/L) but <20 mg/dL (1,200 μmol/L). These infants typically do not excrete phenylketones. Patients with non-PKU hyperphenylalaninemia may still require dietary therapy, depending on their untreated plasma phenylalanine level. Attempts have been made to classify these patients into different subgroups depending on the degree of hyperphenylalaninemia, but the effects of this classification on PKU management and outcomes remain to be clarified. The possibility of deficiency of tetrahydrobiopterin (BH_4) should be investigated in all infants, especially those with milder forms of hyperphenylalaninemia.

Diagnosis

Because of the gradual and nonspecific nature of early clinical symptoms such as vomiting, developmental delay, or eczematoid rash, hyperphenylalaninemia is usually diagnosed through newborn screening in all developed countries. In infants with positive screening results, the diagnosis should be confirmed by quantitative measurement of plasma phenylalanine concentration and molecularly by identifying pathogenic variants in *PAH*. Identification and measurement of phenylketones in the urine have no place in any screening program. In countries and places where such programs are not in effect, identification of phenylketones in the urine by ferric chloride may offer a simple test for the diagnosis of infants with developmental and neurologic abnormalities. All patients with a biochemical diagnosis of hyperphenylalaninemia should undergo pterin measurements in blood or urine to evaluate for defects in BH_4 synthesis or recycling.

Neonatal Screening for Hyperphenylalaninemia

Effective and relatively inexpensive methods for mass screening of newborn infants are used in the United States and many other countries. A few drops of blood, which are placed on a filter paper and mailed to a central laboratory, are used for assay. The screening method of choice uses **tandem mass spectrometry**, which can identify all forms of hyperphenylalaninemia with a low false-positive rate and excellent accuracy and precision. The addition of the phenylalanine:tyrosine molar ratio can improve test specificity and reduce the number of false-positive results. Diagnosis must be confirmed by measurement of plasma phenylalanine concentration and, ultimately by identifying pathogenic variants in *PAH*. Blood phenylalanine in affected infants with PKU may rise to diagnostic levels as early as 4 hours after birth, even in the absence of protein feeding. However, to reduce the number of false-negative results, especially in the milder forms of the condition, it is recommended that the blood for screening be obtained in the first 24-48 hours of life after feeding protein.

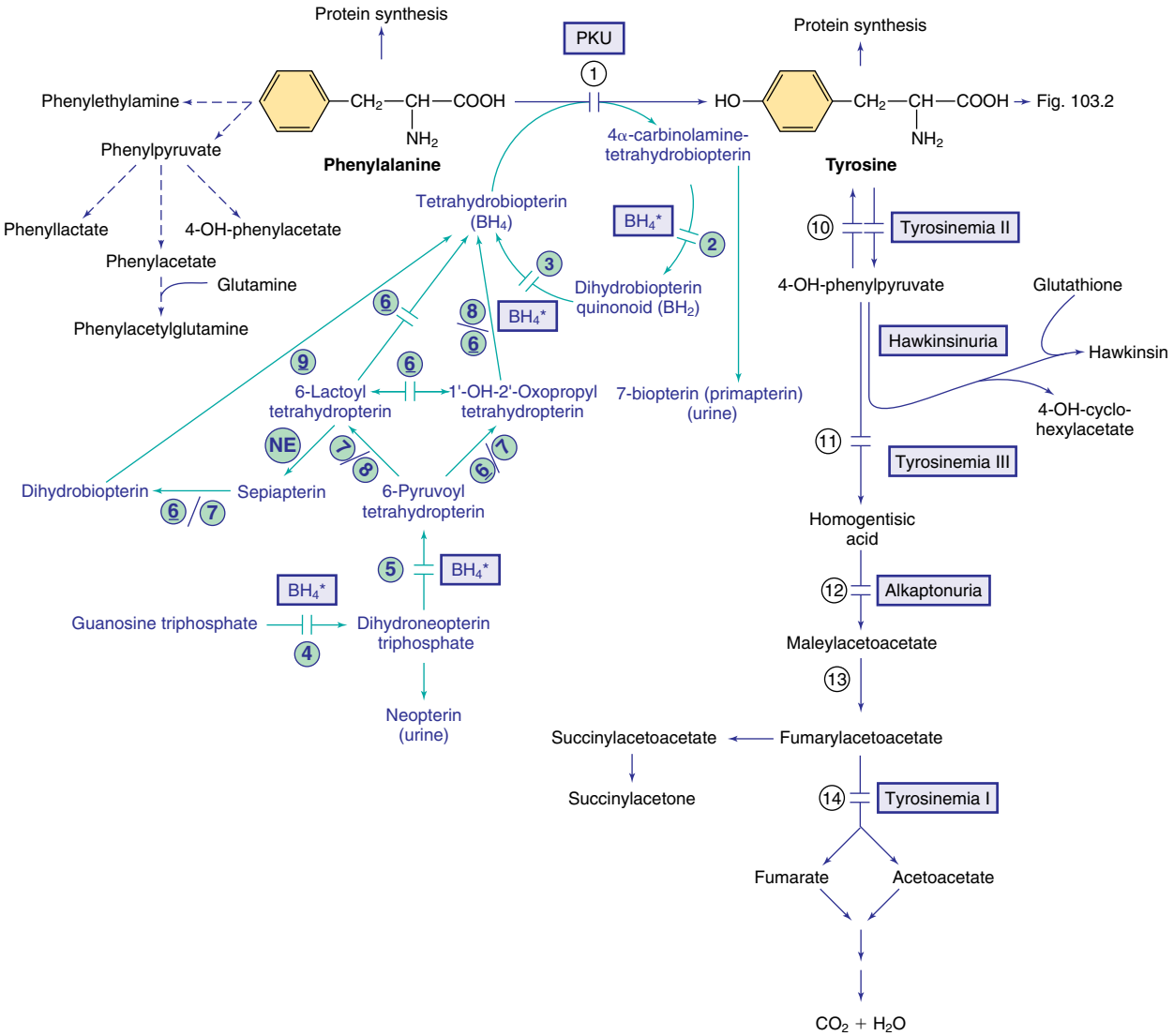

Fig. 105.1 Pathways of phenylalanine and tyrosine metabolism. Enzyme defects causing genetic conditions are depicted as *horizontal bars* crossing the reaction arrow(s). Pathways for synthesis of cofactor BH_4 are shown in *purple*. BH_4* refers to defects of BH_4 metabolism that affect the phenylalanine, tyrosine, and tryptophan hydroxylases (see Figs. 105.2 and 105.5). PKU, Phenylketonuria; NE, nonenzymatic. **Enzymes:** *(1)* Phenylalanine hydroxylase, *(2)* pterin-carbinolamine dehydratase, *(3)* dihydrobiopterin reductase, *(4)* guanosine triphosphate (GTP) cyclohydrolase, *(5)* 6-pyruvoyltetrahydropterin synthase, *(6)* sepiapterin reductase, *(7)* carbonyl reductase, *(8)* aldolase reductase, *(9)* dihydrofolate reductase, *(10)* tyrosine aminotransferase, *(11)* 4-hydroxyphenylpyruvate dioxygenase, *(12)* homogentisic acid dioxygenase, *(13)* maleylacetoacetate isomerase, *(14)* fumarylacetoacetate hydrolase.

Treatment

The mainstay of treatment of PKU is a *low-phenylalanine diet*. The general consensus is to start diet treatment immediately in patients with blood phenylalanine levels >10 mg/dL (600 µmol/L). It is recommended that infants with persistent (more than a few days) plasma levels of phenylalanine ≥6 mg/dL (360 µmol/L) should also be treated with a phenylalanine-restricted diet similar to that in classic PKU. The goal of therapy is to reduce phenylalanine levels in the plasma and brain. Formulas free of or low in phenylalanine are commercially available. The diet should be started as soon as the diagnosis is established. Because phenylalanine is not synthesized endogenously, the diet should provide phenylalanine to prevent phenylalanine deficiency. Dietary phenylalanine tolerance is determined based on age and severity of the PAH deficiency. **Phenylalanine deficiency** is manifested by lethargy, failure to thrive, anorexia, anemia, rashes, diarrhea, and even death. Furthermore, tyrosine can become an essential amino acid in this disorder, and its adequate intake must be ensured. Special food items low in phenylalanine are commercially available for dietary treatment of affected children and adults.

There is no firm consensus concerning optimal levels of blood phenylalanine in affected patients either across different countries or among treatment centers in the United States. The current recommendation is to maintain blood phenylalanine levels between 2 and 6 mg/dL (120 and 360 µmol/L) throughout life. Discontinuation of therapy, even in adulthood, may cause deterioration of neurocognitive performance.

Lifelong adherence to a low-phenylalanine diet is extremely difficult. Patients who maintain good control as children but discontinue the phenylalanine-restricted diet as teenagers or adults may experience significant difficulties with executive function, concentration, emotional liability, and depression. Executive dysfunction may also occur in early-treated children despite diet treatment.

Given the difficulty of maintaining a strict low-phenylalanine diet, there are continuing attempts to find other modalities for treatment of these patients. Administration of **large neutral amino acids** (LNAAs) is one approach to dietary therapy. LNAAs (tyrosine, tryptophan, leucine, isoleucine, valine, methionine, histidine, and phenylalanine) use the same transporter protein (LNAA type 1 or LAT-1) for transit

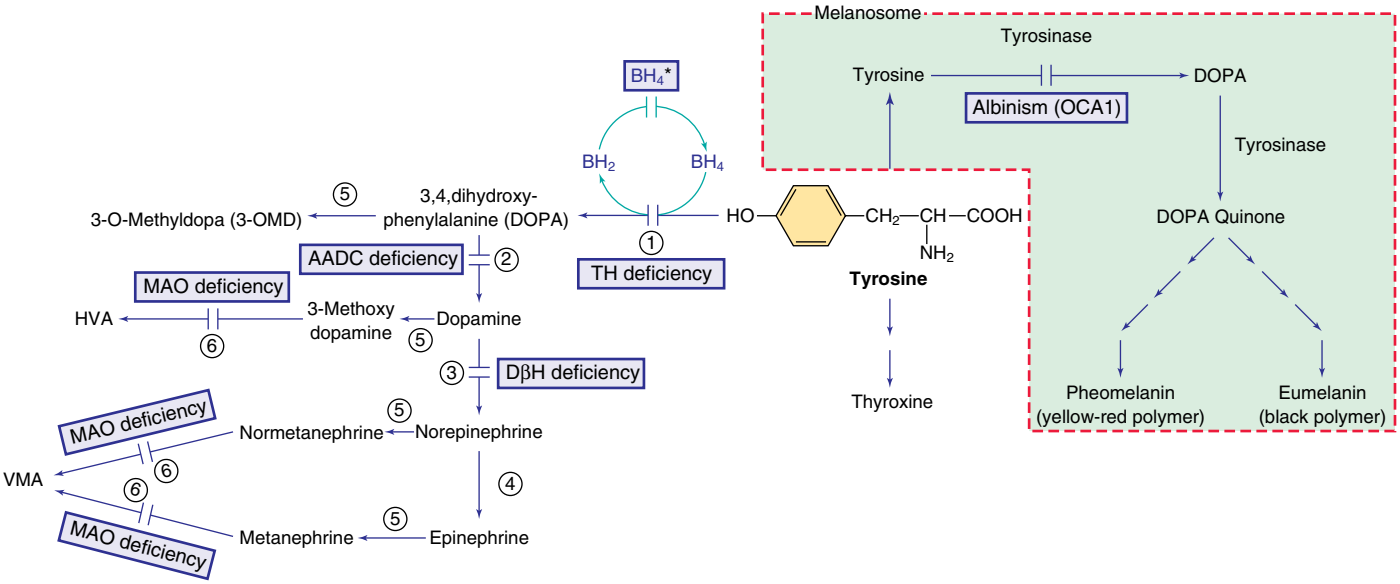

Fig. 105.2 Other pathways involving tyrosine metabolism. BH_4* indicates hyperphenylalaninemia caused by tetrahydrobiopterin (BH_4) deficiency (see Fig. 105.1). HVA, Homovanillic acid; VMA, vanillylmandelic acid. **Enzymes:** *(1)* Tyrosine hydroxylase (TH), *(2)* aromatic L-amino acid decarboxylase (AADC), *(3)* dopamine β-hydroxylase (DβH), *(4)* phenylethanolamine-*N*-methyltransferase (PNMT), *(5)* catechol *O*-methyltransferase (COMT), *(6)* monoamine oxidase (MAO).

through the intestinal cell membrane and BBB. Binding of LNAAs to the transporter is a competitive process. Therefore when LNAAs compete with phenylalanine at the transport level, their large concentrations in the intestinal lumen and blood can reduce the uptake of phenylalanine into the bloodstream and the brain, respectively. Large, controlled clinical trials are necessary to establish the efficacy of this treatment.

Oral administration of BH_4, the cofactor for PAH, may result in the reduction of plasma phenylalanine in some patients with PAH deficiency. Plasma phenylalanine in these patients may decrease enough to allow for considerable modification of their dietary restriction. In very rare cases the diet may be discontinued because the phenylalanine remains under 6 mg/dL (360 µmol/L). The response to BH_4 cannot be predicted consistently based on the genotype alone, especially in compound heterozygous patients. **Sapropterin dihydrochloride**, a synthetic form of BH_4, which acts as a cofactor in patients with residual PAH activity, is approved by the U.S. Food and Drug Administration (FDA) and the European Medicine Agency (EMA) to reduce phenylalanine levels in PKU. A sustained decrease of plasma phenylalanine by at least 30% is consistent with sapropterin responsiveness. Injectable PEGylated recombinant phenylalanine ammonia–lyase has been approved by the FDA and EMA for adult PKU patients with uncontrolled blood phenylalanine levels greater than 600 µmol/L despite prior management.

Pregnancy in Women with PAH Deficiency (Maternal Phenylketonuria)

Pregnant women with PAH deficiency who are not on a phenylalanine-restricted diet have a very high risk of having offspring with intellectual disability, microcephaly, growth retardation, congenital malformations, and congenital heart disease. These complications are directly correlated with elevated maternal blood phenylalanine levels during pregnancy. Prospective mothers who have been treated for PAH deficiency should be maintained on a phenylalanine-restricted diet before and during pregnancy. The best observed outcomes occur when strict control of maternal blood phenylalanine concentration is instituted before pregnancy. Plasma phenylalanine levels >6 mg/dL (360 µmol/L) after conception are associated with increased incidence of intrauterine growth restriction and congenital malformations, as well as lower performance on neurocognitive testing. However, there is strong evidence that phenylalanine control instituted after conception can also result in improved outcomes. The recommended phenylalanine concentration is 2-6 mg/dL (120-360 µmol/L) throughout the pregnancy, although some expert groups advocate plasma phenylalanine levels <4 mg/dL (<240 µmol/L). All women with PAH deficiency who are of childbearing age should be counseled properly regarding the risk of congenital anomalies in their offspring.

HYPERPHENYLALANINEMIA CAUSED BY DEFICIENCY OF THE COFACTOR TETRAHYDROBIOPTERIN

In 1–3% of infants with hyperphenylalaninemia, the defect resides in one of the enzymes necessary for production or recycling of the cofactor BH_4 (see Fig. 105.1). If these infants are misdiagnosed as having PKU, they may deteriorate neurologically despite adequate control of plasma phenylalanine. BH_4 is synthesized from guanosine triphosphate (GTP) through several enzymatic reactions (see Fig. 105.1). In addition to acting as a cofactor for PAH, BH_4 is also a cofactor for tyrosine hydroxylase and tryptophan hydroxylase, which are involved in the biosynthesis of dopamine (see Fig. 105.2) and serotonin (see Fig. 105.5), respectively. Therefore patients with hyperphenylalaninemia resulting from BH_4 deficiency also manifest neurologic findings related to deficiencies of these neurotransmitters. Four enzyme deficiencies leading to defective BH_4 formation cause hyperphenylalaninemia with concomitant deficiencies of dopamine and serotonin: autosomal recessive GTP cyclohydrolase I deficiency (encoded by *GCH1*), 6-pyruvoyl-tetrahydropterin synthase deficiency (encoded by *PTS*), dihydropteridine reductase deficiency (encoded by *QDPR*), and pterin-4-α-carbinolamine dehydratase deficiency (encoded by *PCBD1*). 6-Pyruvoyl-tetrahydropterin synthase is the most frequent cause of hyperphenylalaninemia-associated BH_4 deficiency. Autosomal dominant forms of GTP cyclohydrolase I deficiency and sepiapterin reductase deficiency result in deficiencies of neurotransmitters without hyperphenylalaninemia (see Chapter 105.11).

Clinical Manifestations

Infants with cofactor BH_4 deficiency are identified during screening programs for PKU because of hyperphenylalaninemia. Plasma phenylalanine levels may be as high as those in classic PKU or may be in the milder range. However, clinical manifestations of the neurotransmitter disorders differ greatly from those of PKU. Neurologic symptoms of the neurotransmitter disorders often manifest in the first few months of life and include extrapyramidal signs (choreoathetotic or dystonic limb movements, axial and truncal hypotonia, hypokinesia), feeding

difficulties, and autonomic abnormalities. Intellectual disability, seizures, hypersalivation, and swallowing difficulties can also be seen. The symptoms are usually progressive and often have a marked diurnal fluctuation. Prognosis and outcome strongly depend on the age of diagnosis, treatment, and the underlying enzyme defect.

Diagnosis

Despite the low incidence of BH_4 synthesis and recycling defects, all newborns with hyperphenylalaninemia detected through newborn screening *must* be screened for BH_4 synthesis defects. BH_4 deficiency and the responsible enzyme defect may be diagnosed by several studies.

Measurement of Neopterin and Biopterin

Neopterin (an oxidative product of dihydroneopterin triphosphate) and biopterin (an oxidative product of dihydrobiopterin and BH_4) are measured in body fluids, especially urine (see Fig. 105.1). In patients with the autosomal recessive form of GTP cyclohydrolase I deficiency, urinary excretion of both neopterin and biopterin is reduced and is often very low. In patients with 6-pyruvoyl-tetrahydropterin synthase deficiency, there is a marked elevation of neopterin excretion and a concomitant decrease in biopterin excretion. Patients with pterin-4-α-carbinolamine dehydratase deficiency can be identified by detecting urinary primapterin (an isomer of biopterin). In dihydropteridine reductase deficiency, no consistent pattern in the excretion of neopterin and biopterin has been observed, thus necessitating enzymatic studies of DHPR in erythrocytes and/or molecular confirmation through *QDPR* gene analysis.

Cerebrospinal Fluid Studies

Examination of cerebrospinal fluid (CSF) may reveal decreased levels of dopamine and serotonin metabolites (see Chapter 105.11).

BH_4 Loading Test

An oral dose of BH_4 (20 mg/kg) normalizes plasma phenylalanine and the phenylalanine:tyrosine ratio in patients with BH_4-responsive deficiency within 4-12 hours. The baseline blood phenylalanine should be elevated (>400 μmol/L) to enable interpretation of the results. This may be achieved by discontinuing diet therapy for 2 days before the test. In BH_4-responsive PAH deficiency, blood phenylalanine levels may decrease during the BH_4 loading test but increase later, even with BH_4 supplementation. Patients who demonstrate phenylalanine levels within the normal range over at least 1 week without a phenylalanine-restricted diet can continue BH_4 supplementation as the sole treatment for the hyperphenylalaninemia. However, it is imperative that plasma phenylalanine levels be monitored prospectively to ensure that phenylalanine levels remain within the normal range.

Molecular Testing

Sequencing and deletion/duplication analysis are clinically available and have an important role in confirming the biochemical diagnosis of PAH deficiency and of the BH_4 synthesis and recycling disorders.

Enzyme Assay

The activity of dihydropteridine reductase can be measured in the dry blood spots on the filter paper used for screening purposes or in erythrocytes. 6-Pyruvoyl-tetrahydropterin synthase activity can be measured in liver tissue, fibroblasts, and erythrocytes. Pterin-4-α-carbinolamine dehydratase activity can be measured in liver tissue and fibroblasts. GTP cyclohydrolase I activity can be measured in the liver and in cytokine (interferon-γ)–stimulated mononuclear cells or fibroblasts (the enzyme activity is normally very low in unstimulated cells). Molecular testing offers a more convenient method to secure the diagnosis in this group of disorders.

Treatment

The goals of therapy are to correct hyperphenylalaninemia and to restore neurotransmitter deficiencies in the CNS. Control of hyperphenylalaninemia is important in patients with cofactor deficiency because high levels of phenylalanine cause intellectual disability and interfere with the transport of neurotransmitter precursors (tyrosine and tryptophan) into the brain. Plasma phenylalanine should be maintained as close to normal as possible (<6 mg/dL or <360 μmol/L). This can be achieved by oral supplementation of BH_4 (5-20 mg/kg/day). Sapropterin dihydrochloride, the synthetic form of BH_4, is commercially available but expensive. In patients receiving dietary interventions, phenylalanine and tyrosine deficiencies should be avoided.

Lifelong supplementation with neurotransmitter precursors such as L-dopa and 5-hydroxytryptophan, along with carbidopa to inhibit degradation of L-dopa before it enters the CNS, is necessary in most of these patients even when treatment with BH_4 normalizes plasma levels of phenylalanine. BH_4 does not readily enter the brain to restore neurotransmitter production. To minimize untoward side effects (especially L-dopa–induced dyskinesia), the treatment should be started with low doses of L-dopa/carbidopa and 5-hydroxytryptophan. The treatment should be gradually adjusted based on response to therapy and clinical improvement for each individual patient. Supplementation with folinic acid is also recommended in patients with dihydropteridine reductase deficiency. Unfortunately, attempting to normalize neurotransmitter levels using neurotransmitter precursors usually does not fully resolve the neurologic symptoms because of the inability to attain normal levels of BH_4 in the brain. Patients often demonstrate intellectual disability, fluctuating abnormalities of tone, eye movement abnormalities, poor balance and coordination, decreased ability to ambulate, and seizures despite supplementation with neurotransmitter precursors.

Hyperprolactinemia occurs in patients with BH_4 deficiency and may be the result of hypothalamic dopamine deficiency. Measurement of serum prolactin levels may be a convenient method for monitoring adequacy of neurotransmitter replacement in affected patients.

Some drugs, such as trimethoprim/sulfamethoxazole, methotrexate, and other antileukemic agents, are known to inhibit dihydropteridine reductase enzyme activity and should be used with great caution in patients with BH_4 deficiency.

Genetics and Prevalence

All defects causing hyperphenylalaninemia are inherited as autosomal recessive traits. Autosomal dominant forms of GTP cyclohydrolase I deficiency and sepiapterin reductase deficiency result in neurotransmitter disorders without hyperphenylalaninemia (see Chapter 105.11). The prevalence of PKU in the United States is estimated at 1 in 14,000 to 1 in 20,000 live births. The prevalence of non-PKU hyperphenylalaninemia is estimated at 1 in 50,000 live births. Most patients are compound heterozygotes for two different pathogenic variants.

TETRAHYDROBIOPTERIN DEFECTS WITHOUT HYPERPHENYLALANINEMIA

See Chapter 105.11.

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105.2 Tyrosine

Oleg A. Shchelochkov and Charles P. Venditti

Tyrosine is derived from ingested proteins or is synthesized endogenously from phenylalanine. It is used for protein synthesis and as a precursor of dopamine, norepinephrine, epinephrine, melanin, thyroxine, and quinoproteins. Excess tyrosine is metabolized to carbon dioxide and water through the tyrosine degradation pathway (see Fig. 105.1). Hereditary causes of hypertyrosinemia include deficiencies of the enzymes fumarylacetoacetate hydrolase **(FAH)**, tyrosine aminotransferase **(TAT)**, and 4-hydroxyphenylpyruvate dioxygenase **(4-HPPD)**. **Acquired hypertyrosinemia** may occur in severe hepatocellular dysfunction (liver failure), scurvy (vitamin C is a cofactor for 4-HPPD), and hyperthyroidism. Hypertyrosinemia is common in blood samples obtained soon after eating and in premature infants.

TYROSINEMIA TYPE I (FUMARYLACETOACETATE HYDROLASE DEFICIENCY, HEPATORENAL TYROSINEMIA)

Tyrosinemia type I is a severe multisystemic disease caused by FAH deficiency. Liver, kidney, and nerve damage is likely caused by metabolites of tyrosine degradation, especially fumarylacetoacetate and succinylacetone.

Clinical Manifestations and Natural History

Affected infants may appear healthy at birth but develop symptoms in the first year of life. Most patients present between 2 and 6 months of age but rarely may become symptomatic in the first month or appear unaffected beyond the first year of life. Earlier presentation confers poorer prognosis. In untreated children, a 1-year mortality can approach 60% if infants develop symptoms before 2 months of age and decrease to 4% in infants who become symptomatic after 6 months.

An acute **hepatic crisis** typically heralds the disease onset and is usually precipitated by an intercurrent illness leading to a catabolic state. Fever, irritability, vomiting, hemorrhage, hepatomegaly, jaundice, elevated levels of serum transaminases, hypoglycemia, and neuropathy are common. An odor resembling boiled cabbage resulting from increased methionine metabolites may be present. Without treatment, hepatic crises may progress to liver failure and death. Between the crises, varying degrees of failure to thrive, hepatomegaly, and coagulation abnormalities often persist. Cirrhosis and eventually hepatocellular carcinoma occur with increasing age.

Episodes of acute **peripheral neuropathy** resembling acute porphyria occur in approximately 40% of affected children. These crises, often triggered by a minor infection, are characterized by severe pain, often in the legs, associated with extensor hypertonia of the neck and trunk, vomiting, paralytic ileus, and occasionally self-induced injuries of the tongue or buccal mucosa. Marked weakness occurs in about 30% of episodes, which may lead to respiratory failure requiring mechanical ventilation. Crises typically last 1-7 days, but recuperation from paralytic crises can take weeks to months.

Renal involvement manifests as a Fanconi renal syndrome with hyperphosphaturia, hypophosphatemia, normal–anion gap metabolic acidosis, and vitamin D–resistant rickets. Nephromegaly and nephrocalcinosis may be present on ultrasound examination. Glomerular abnormalities can be observed in adolescents and older patients.

Hypertrophic cardiomyopathy and hyperinsulinism are seen in some infants.

Laboratory Findings

Elevated levels of succinylacetone in serum and urine are diagnostic for tyrosinemia type I (see Fig. 105.1). Succinylacetone levels may fall below the diagnostic threshold in patients treated with nitisinone. In untreated patients, serum α-fetoprotein is increased, often greatly, and liver-synthesized coagulation factors are decreased in most patients. Increased levels of α-fetoprotein are present in the cord blood of affected infants, indicating liver damage beginning prenatally. Serum transaminase levels are often increased, with marked increases during acute hepatic episodes. Serum concentration of bilirubin is usually normal but can be increased in severe liver failure. Plasma tyrosine levels are usually elevated at diagnosis, but this is a nonspecific finding and can vary depending on dietary intake. Plasma levels of other amino acids, particularly methionine, may also be elevated in patients with liver damage. Hyperphosphaturia, hypophosphatemia, and generalized aminoaciduria may occur. Urinary 5-aminolevulinic acid (also known as *delta-aminolevulinic acid*) is elevated due to the inhibition of 5-aminolevulinate dehydratase by succinylacetone (see Fig. 112.1).

Diagnosis is usually established by demonstration of elevated succinylacetone in urine or blood. Neonatal screening for hypertyrosinemia using tyrosine alone detects only a fraction of patients with tyrosinemia type I. Succinylacetone assayed by many neonatal screening programs has higher sensitivity and specificity than tyrosine and is the preferred metabolite for screening. Tyrosinemia type I should be differentiated from other causes of hepatitis and hepatic failure in infants, including galactosemia, hereditary fructose intolerance, neonatal iron storage disease, giant cell hepatitis, citrullinemia type II, citrin deficiency, and hemophagocytic lymphohistiocytosis (see Chapter 105.12).

Treatment and Outcome

A diet low in phenylalanine and tyrosine can slow, but not halt, the progression of the condition. The treatment of choice is **nitisinone** (NTBC), which inhibits 4-HPPD, reduces the flux of tyrosine metabolites to FAH, and decreases the production of the offending compounds fumarylacetoacetate and succinylacetone (see Fig. 105.1). The dose of nitisinone is titrated to the lowest, most effective dose (usually targeting the blood range of 20-40 μmol/L), aiming to suppress succinylacetone production while maintaining a plasma tyrosine level <400 μmol/L (7.2 mg/dL). This treatment with nitisinone can prevent acute hepatic and neurologic crises. Although nitisinone greatly slows disease progression, pretreatment liver damage cannot be completely reversed. Therefore patients must be followed for the development of cirrhosis or hepatocellular carcinoma. On imaging, the presence of even a single liver nodule usually indicates underlying cirrhosis. Most liver nodules in FAH-deficient patients are benign, but current imaging techniques cannot accurately distinguish all malignant nodules. For patients with severe liver failure not responding to nitisinone, liver transplantation is an effective therapy, which can also alleviate the risk of hepatocellular carcinoma. The impact of nitisinone treatment on liver transplantation is under study, but the greatest positive effect is seen in patients treated early, such as children detected by neonatal screening, before developing clinical symptoms. In early-treated patients, nitisinone has greatly reduced the need for liver transplantation. Because nitisinone treatment can increase plasma tyrosine, a low-tyrosine, low-phenylalanine diet is recommended. Rarely, nitisinone-treated patients develop corneal crystals, presumably composed of tyrosine, but these tend to be reversible with strict dietary compliance. This finding, combined with observations of developmental delay in some patients with tyrosinemia type II who chronically have elevated tyrosine levels, suggest that a diet low in phenylalanine and tyrosine should be continued in patients treated with nitisinone. The dietary treatment of patients with tyrosine and phenylalanine restriction necessitates surveillance of growth and development by ensuring adequate intake of amino acids and other nutrients.

Genetics and Prevalence

Tyrosinemia type I is inherited as an autosomal recessive trait and is caused by biallelic pathogenic variants in *FAH*. DNA analysis is useful for carrier testing in groups at risk for specific pathogenic variants, such as French Canadians from the Saguenay-Lac Saint-Jean region of Quebec. The prevalence of tyrosinemia type I is estimated to be 1 in 1,846 live births in the Saguenay-Lac Saint-Jean region and approximately 1 in 100,000 live births worldwide. Prenatal screening can be performed by measurement of succinylacetone in amniotic fluid.

TYROSINEMIA TYPE II (TYROSINE AMINOTRANSFERASE DEFICIENCY, RICHNER-HANHART SYNDROME, OCULOCUTANEOUS TYROSINEMIA)

Tyrosinemia type II is a rare autosomal recessive disorder caused by deficiency of cytosolic tyrosine aminotransferase that results in palmar and plantar hyperkeratosis, herpetiform corneal ulcers, and intellectual disability (see Fig. 105.1). *Ocular manifestations,* which may occur as early as 6 months of age, include excessive tearing, redness, pain, and photophobia. Corneal lesions are presumed to be caused by tyrosine crystals deposition. In contrast to herpetic ulcers, corneal lesions in tyrosinemia type II stain poorly with fluorescein and often are bilateral. *Skin lesions,* which may develop later in life, include painful, nonpruritic hyperkeratotic plaques on the soles, palms, and fingertips. Intellectual disability, which occurs in approximately 50% of patients, is usually mild to moderate. The contribution of consanguinity in this rare disorder is incompletely understood.

The principal **laboratory finding** in untreated patients is marked hypertyrosinemia, >500 μmol/L, and it may reach 1,100-2,750 μmol/L. Surprisingly, in some patients urinary 4-hydroxyphenylpyruvic acid and its metabolites can be elevated despite them being downstream

from the metabolic block (see Fig. 105.1). It is hypothesized that other transaminases in the presence of high tyrosine concentrations can produce 4-hydroxyphenylpyruvic acid in mitochondria, where it cannot be further degraded. In contrast to tyrosinemia type I, liver and kidney function tend to be normal, as are serum concentrations of other amino acids and succinylacetone. Tyrosinemia type II is caused by tyrosine aminotransferase *(TAT)* gene pathogenic variants, causing a deficiency of cytosolic TAT activity in the liver.

Diagnosis of type II tyrosinemia is established by an assay of plasma tyrosine concentration in patients with suggestive findings and can be confirmed molecularly. An assay of hepatic TAT requires a liver biopsy and is rarely indicated.

Treatment with a diet low in tyrosine and phenylalanine, aiming to achieve plasma tyrosine levels <500 μmol/L, helps improve skin and eye manifestations. Early dietary intervention can lead to improved intellectual outcomes.

TYROSINEMIA TYPE III (PRIMARY DEFICIENCY OF 4-HYDROXYPHENYLPYRUVATE DIOXYGENASE)

Tyrosinemia type III is an autosomal recessive condition caused by biallelic pathogenic variants in *HPD* encoding the enzyme 4-HPPD. Only a few patients with tyrosinemia type III have been reported. Most were detected by amino acid chromatography performed for various neurologic findings. Therefore ascertainment bias likely confounds current understanding of this disorder. Some asymptomatic infants with 4-HPPD deficiency have been identified by neonatal screening for hypertyrosinemia. Age at presentation varied between 1 and 17 months. In symptomatic patients, developmental delay, seizures, intermittent ataxia, and self-injurious behavior have been reported. Liver and renal abnormalities tend to be absent.

The role of 4-HPPD deficiency in the disease needs further study. The **diagnosis** is suspected in children with sustained moderate increases in plasma levels of tyrosine (typically 350-700 μmol/L on a normal diet) and the presence of 4-hydroxyphenylpyruvic acid and its metabolites, urinary 4-hydroxyphenyllactate and 4-hydroxyphenylacetate. The diagnosis should be confirmed by demonstrating biallelic pathogenic variants in *HPD* or, rarely, by demonstrating a low activity of the 4-HPPD enzyme. The latter requires a liver biopsy and is not usually indicated.

Given a possible association with neurologic abnormalities and corneal deposition of tyrosine crystals, dietary control of plasma tyrosine levels is recommended. One could also consider a trial with vitamin C, the cofactor for 4-HPPD, which can help lower plasma tyrosine in some patients.

HAWKINSINURIA

Hawkinsinuria is a condition allelic to tyrosinemia type III (4-HPPD deficiency). Some specific missense variants (e.g., p.Ala33Thr or p.Asn241Ser) in *HPD* that encode 4-HPPD can result in uncoupling of typical oxidization of 4-hydroxyphenylpyruvate to homogentisic acid and lead to premature release of quinolacetic acid. The abnormal enzyme, incapable of normally oxidizing 4-hydroxyphenylpyruvate to homogentisic acid, forms an intermediate that reacts with glutathione to form the unusual organic acid **hawkinsin** ([2-L-cystein-*S*-yl-1,4-dihydroxycyclohex-5-en-1-yl]acetic acid, see Fig. 105.1). As a result, secondary glutathione deficiency may ensue. Hawkinsinuria is inherited as an autosomal dominant trait. In one patient, compound heterozygosity for alleles associated with hawkinsinuria and tyrosinemia type III produced biochemical features of hawkinsinuria.

The clinical course of this rare disorder is incompletely understood. Individuals with hawkinsinuria may present with symptoms only during infancy. The symptoms usually appear in the first few months of life, typically after weaning from breastfeeding and with the introduction of a high-protein diet. Severe metabolic acidosis, ketosis, failure to thrive, anemia, mild hepatomegaly, renal tubular acidosis, and an unusual odor are reported manifestations of this disorder. Neurocognitive development is usually normal.

Symptomatic infants and asymptomatic affected children and adults excrete hawkinsin, 4-hydroxyphenylpyruvate, and its metabolites (4-hydroxyphenyllactate and 4-hydroxyphenylacetate) as well as 4-hydroxycyclohexylacetate and 5-oxoproline (due to secondary glutathione deficiency) in their urine. Plasma tyrosine is moderately elevated in symptomatic infants and may normalize in asymptomatic older patients.

Treatment consists of a low-protein diet during infancy. Breastfeeding is encouraged. Successful long-term use of *N*-acetyl-L-cysteine to treat secondary glutathione deficiency has been reported. A trial with vitamin C is recommended. Although the affected enzyme is susceptible to inhibition by nitisinone, clinical studies showing its efficacy in symptomatic infants are lacking, and the indications for its use are not known.

TRANSIENT TYROSINEMIA OF THE NEWBORN

In a small number of newborn infants, plasma tyrosine may be as high as 3,300 μmol/L during the first 2 weeks of life. Most affected infants are premature and are on a high-protein diet. Transient tyrosinemia is thought to result from delayed maturation of 4-HPPD (see Fig. 105.1). Lethargy, poor feeding, and decreased motor activity are noted in some patients. Most are asymptomatic and are identified by a high blood phenylalanine or tyrosine level on routine newborn screening. Laboratory findings include marked elevation of plasma tyrosine with a moderate increase in plasma phenylalanine. The finding of hypertyrosinemia differentiates this condition from PKU. 4-Hydroxyphenylpyruvic acid and its metabolites are present in the urine. Hypertyrosinemia tends to resolve spontaneously in the first 2 months of life and can be improved by reducing dietary protein to below 2 g/kg/day and by administering vitamin C. Mild intellectual deficits have been reported in some infants who had this condition, but the causal relationship to hypertyrosinemia has not been conclusively established.

ALKAPTONURIA

Alkaptonuria is a rare (approximately 1 in 250,000 live births) autosomal recessive disorder caused by a deficiency of homogentisate 1,2-dioxigenase encoded by *HGD*. Large amounts of homogentisic acid are formed (see Fig. 105.1) and excreted in urine or deposited in tissues. Alkaptonuria has a worldwide distribution, with a higher disease prevalence in the Dominican Republic and Slovakia.

The main **clinical manifestations** of alkaptonuria consist of ochronosis and arthritis in adulthood. The only sign in children is blackening of the urine on standing, caused by oxidation and polymerization of homogentisic acid. A history of gray- or black-stained diapers should suggest the diagnosis. This sign may never be noted, thus delaying the diagnosis until adulthood. *Ochronosis,* seen clinically as dark spots on the sclera or ear cartilage, results from the accumulation of the black polymer of homogentisic acid. *Arthritis* is another result of this deposition and can be disabling with advancing age. It can involve the spine and large joints (shoulders, hips, and knees) and is usually more severe in males. As with rheumatoid arthritis, the alkaptonuric arthritis has acute exacerbations, but its radiologic findings are more typical of osteoarthritis, with characteristic narrowing of the joint spaces and calcification of the intervertebral disks. A high incidence of heart disease (mitral and aortic valvulitis, calcification of heart valves, myocardial infarction) has been reported.

The **diagnosis** is confirmed by finding massive excretion of homogentisic acid on urine organic acid testing. Tyrosine levels are normal. The enzyme is expressed in high levels only in the liver and kidneys.

Treatment of the arthritis is symptomatic. In clinical trials, nitisinone, an inhibitor of 4-HPPD, has been shown to reduce urinary homogentisic acid. Additional studies are needed to establish its clinical efficacy and safety profile.

TYROSINE HYDROXYLASE DEFICIENCY

See Chapter 105.11.

ALBINISM

See also Chapters 662 and 694.

Albinism is caused by impaired synthesis and distribution of **melanin**, the main pigment of the skin and eye (Table 105.1). Melanin is synthesized by melanocytes from tyrosine in a membrane-bound intracellular organelle, the melanosome. Melanocytes

Table 105.1 Classification of Major Causes of Albinism

TYPE	GENE	DISTINGUISHING MOLECULAR AND CLINICAL FEATURES
OCULOCUTANEOUS ALBINISM (OCA)		
OCA1A (severe deficiency)	*TYR*	OCA1A (no melanin synthesis) vs OCA1B (minimal amounts of melanin present). OCA1A results in poorer visual outcomes.
OCA2 (tyrosinase positive)	*OCA2*	Newborns typically have light pigment, and hair color can darken with age.
OCA3 (Rufous, red OCA)	*TYRP1*	TYRP1 is required for maturation of eumelanin, but not reddish pheomelanin.
OCA4	*SLC45A2*	Newborns typically have light pigment, and hair color can darken with age. Clinically, it tends to overlap with OCA2.
OCA6	*SLC24A5*	*SLC24A5* encodes the protein NCKX5 involved in the maturation of melanosomes.
OCA7	*LRMDA*	Tends to be associated with poorer visual outcomes.
OCA8	*DCT*	*DCT* encodes dopachrome tautomerase involved in the melanin synthesis upstream of TYRP1. Likely results in milder forms of OCA.
Hermansky-Pudlak syndrome	Multiple genes	A multisystemic, genetically heterogenous disorder characterized by OCA, bleeding tendency, interstitial pneumonia, and granulomatous colitis, among other complications.
Chédiak-Higashi syndrome	*LYST*	Results in partial OCA, bleeding tendency, immunodeficiency, and hemophagocytic lymphohistiocytosis.
OCULAR ALBINISM (OA)		
OA1 (Nettleship-Falls ocular albinism)	*GPR143*	X-linked inheritance. Affected males present with significant ocular manifestations with minor skin involvement.
LOCALIZED ALBINISM		
Piebaldism	*KIT* and *SNAI2*	Pathogenic variants in *KIT* have been associated with a number of phenotypes, including piebaldism, mastocytosis, familial, and sporadic cancers.
Waardenburg syndrome (WS1-WS4)	See text.	See text.

originate from the embryonic neural crest and migrate to the skin, eyes (choroid and iris), hair follicles, and inner ear. The melanin in the eye is confined to the iris stromal and retinal pigment epithelia, whereas in skin and hair follicles, it is secreted into the epidermis and hair shaft. Albinism can be caused by deficiencies of melanin synthesis, by some hereditary defects of melanosomes, or by disorders of melanocyte migration. Neither the biosynthetic pathway of melanin nor many facets of melanocyte cell biology are completely elucidated (see Fig. 105.2). The end products are two pigments: *pheomelanin,* which is a yellow-red pigment, and *eumelanin,* a brown-black pigment.

Clinically, primary albinism can be *generalized* or *localized.* Primary generalized albinism can be *ocular* or *oculocutaneous.* Some syndromes feature albinism in association with platelet, immunologic, or neurologic dysfunction. In generalized oculocutaneous albinism, hypopigmentation can be either complete or partial. Individuals with complete albinism do not develop either generalized (tanning) or localized (pigmented nevi) skin pigmentation.

The **diagnosis** of albinism is usually evident, but for some children whose families are particularly light-skinned, normal variation may be a diagnostic consideration. Unlike patients with albinism, unaffected fair-skinned children progressively develop pigmentation with age, do not exhibit the eye manifestations of albinism, and have pigmentary development similar to other family members. The clinical diagnosis of oculocutaneous albinism, as opposed to other types of cutaneous hypopigmentation, requires the presence of characteristic eye findings.

The **ocular manifestations** of albinism include hypopigmentation of the iris and retina, often with foveal hypoplasia, along with reduced visual acuity, refractive errors, nystagmus, alternating strabismus, and iris transillumination (diffuse reddish hue of the iris produced during ophthalmoscopic or slit-lamp examination of the eye).

Albinism is also associated with an abnormality in routing optic fibers at the chiasm. Unlike in pigmented individuals, in patients with albinism, the majority of nerve fibers from the temporal side of the retina cross to the contralateral hemisphere of the brain. This results in impaired depth perception, alternating strabismus, and a characteristic pattern of visual evoked potentials. These findings are highly specific for albinism. Regular ophthalmologic follow-up is recommended for patients with oculocutaneous albinism. Correction of refractive errors can maximize visual function. Usually, the alternating strabismus does not result in amblyopia and does not require surgery.

Patients with albinism should be counseled to avoid ultraviolet (UV) radiation by wearing protective long-sleeved clothing and by using sunscreens with a sun protection factor (SPF) rating >30. Melanin is also present in the cochlea; therefore albino individuals may be more susceptible to ototoxic agents such as gentamicin.

Genetics of Albinism

Oculocutaneous albinism is inherited as an autosomal recessive trait. Many clinical forms of albinism have been identified. Some of the seemingly distinct clinical forms are caused by different pathogenic variants of the same gene. Implicated genes are involved in different stages of melanogenesis and melanosome maturation (see Table 105.1). The classification outlined next is based on the distribution of albinism in the body and the affected genes. Genetic analysis is clinically available for most forms of albinism (see Table 105.1). Molecular diagnosis is of little use therapeutically in isolated albinism but can be helpful for precise genetic counseling of families.

Oculocutaneous (Generalized) Albinism

Lack of pigment is generalized, affecting skin, hair, and eyes. At least eight genetically distinct forms of oculocutaneous albinism **(OCA)** have been identified of which seven have pathogenic variants in

associated genes (OCA1, OCA2, OCA3, OCA4, OCA6, OCA7, and OCA8). Many forms of OCA may not be clinically distinguishable from one another. All affected individuals have ocular manifestations of albinism. All known forms of OCA are inherited as autosomal recessive traits.

OCA1

The defect in patients with OCA1 resides in the tyrosinase gene, *TYR*. Many pathogenic variants have been identified. Most affected individuals are compound heterozygotes. A clinical clue to the diagnosis of OCA1 is complete lack of pigment at birth. The condition can be subdivided into OCA1A and OCA1B, based on enzyme activity and difference in clinical manifestations as a function of age. In patients with OCA1A, the most severe form of OCA, both *TYR* alleles have pathogenic variants that completely inactivate tyrosinase. Clinically, lack of pigment in the skin (milky white), hair (white hair), and eyes (red-gray irides) is evident at birth and remains unchanged throughout life. They do not tan and do not develop pigmented nevi or freckles.

Patients with OCA1B have *TYR* gene pathogenic variants that preserve some residual activity. Clinically, they completely lack pigment at birth but with age become light blond with light-blue or hazel eyes. They develop pigmented nevi and freckles, and they may tan.

OCA2

OCA2 is the *most common form of generalized OCA,* particularly in patients of African ancestry. Clinically, the phenotype is highly variable; most patients demonstrate some pigmentation of the skin and eyes at birth and continue to accumulate pigment throughout life. The hair is yellow at birth and may darken with age. They have pigmented nevi and freckles, and some may tan. They may be clinically indistinguishable from OCA1B patients. Individuals with OCA2, however, have normal tyrosinase activity in hair bulbs. The defect is in the *OCA2* gene, which is an orthologue of the *p* (pink-eyed dilution) gene in the mouse. This gene produces the P protein, a melanosome membrane–bound protein. Patients with **Prader-Willi** and **Angelman** syndromes caused by microdeletion of chromosome 15q12 that includes the *OCA2* gene have mild pigmentary deficiency (see Chapter 98.8).

OCA3

This form has been identified predominantly in patients of African and Asian ancestry. Patients with OCA3 can make pheomelanin but not eumelanin. Patients have reddish hair and reddish-brown skin as adults. The skin color is peculiar to this form. In young persons the coloration may resemble that of OCA2. The altered gene *TYRP1* encodes the tyrosinase-related protein 1, the function of which is not well-understood.

OCA4

Similar manifestations to OCA2 (both in the skin and the eyes) have been observed in OCA4 patients (mostly from Japan) with pathogenic variants in the *SLC45A2* gene.

Ocular Albinism

Patients with ocular albinism **(OA)** present in the first months of life with nystagmus, hypopigmentation of the iris and fundus, foveal hypoplasia, and decreased visual acuity. Electron microscopy demonstrates characteristic macromelanosomes in skin biopsies or hair root specimens. Most patients affected by OA have ocular albinism type 1 **(OA1)**, an X-linked disorder caused by pathogenic variants in the *GPR143* gene. A rare form of OA with late-onset sensorineural deafness and apparent autosomal dominant inheritance has also been reported.

Ocular Albinism Type 1 (Nettleship-Falls Ocular Albinism)

OA1 is an X-linked disorder characterized by congenital nystagmus, reduced pigmentation of ocular structures, and visual impairment in affected males. Heterozygous females may present with segments of abnormal retinal pigmentations. Infrequently, depending on the pattern of X chromosome inactivation, heterozygous females may also present with severe manifestations, including nystagmus, iris and foveal hypopigmentation, foveal hypoplasia, and reduced visual acuity. In families with darker skin complexion, mild skin hypopigmentation can be seen. The diagnosis of OA1 is suspected in males with features of albinism in the eye, normal to mildly reduced skin pigmentation, and a family history suggestive of an X-linked transmission. It is a nonprogressive disorder, and the eye findings often improve with age. In patients who are the first of their families to be affected, genetic analysis of *GPR143* helps confirm the diagnosis.

Syndromic Forms of Generalized Albinism

Hermansky-Pudlak Syndrome

This group of autosomal recessive disorders is caused by biallelic pathogenic variants in one of ten genes *(HPS1, AP3B1, HPS3, HPS4, HPS5, HPS6, DTNBP1, BLOC1S3, BLOC1S6, AP3D1)*. Hermansky-Pudlak syndrome **(HPS)** can be suspected in patients with albinism associated with a bleeding diathesis, inflammatory bowel disease (IBD), and/or pulmonary fibrosis. Disease subtype can be established with molecular studies (see Chapter 533). Genes associated with HPS are required for normal structure and function of lysosome-derived organelles, including melanosomes and platelet-dense bodies. Patients have a tyrosinase-positive OCA of variable severity associated with platelet dysfunction (caused by the absence of platelet-dense bodies). A ceroid-like material accumulates in tissues. HPS is pan-ethnic. Patient ancestry can help develop a cost-effective testing strategy. HPS is prevalent in two regions of Puerto Rico (**type 1** in the northwest and **type 3** in the central regions because of different founder effects). The cutaneous and ocular symptoms of albinism are present. Patients can develop epistaxis, postsurgical bleeding, or abundant menses. Bleeding time is prolonged, but platelet count is normal. Major complications include progressive pulmonary fibrosis in young adults and Crohn's-like IBD in adolescents and young adults. Kidney failure and cardiomyopathy have been reported. Neutropenia has been described in HPS **type 2**. Treatment is symptomatic.

Chédiak-Higashi Syndrome

Patients with this rare autosomal recessive condition have OCA of variable severity and susceptibility to infection (see Chapter 156). Bacterial infections of the skin and the upper respiratory tract are common. Giant peroxidase-positive lysosomal granules can be seen in granulocytes in a blood smear. Patients have a reduced number of melanosomes, which are abnormally large (macromelanosomes) and can have silvery-gray hair. The bleeding tendency is typically mild. If treatment is not successful, children can reach a stage of the disease known as the **accelerated phase**, which is a major, life-threatening complication of Chédiak-Higashi syndrome. It is caused by macrophage activation resulting in hemophagocytic lymphohistiocytosis with manifestations that include fever, lymphadenopathy, hepatosplenomegaly, cytopenia, and an elevated plasma ferritin level. Patients surviving childhood may develop cerebellar atrophy, peripheral neuropathy, and cognitive delay. Pathogenic variants in *LYST* are the only known cause for this syndrome. Hematopoietic stem cell transplantation offers an effective approach to control immunodeficiency and hematologic abnormalities and to prevent development of the accelerated phase.

Other Disorders Featuring Generalized Albinism

Hypopigmentation can be a feature in other syndromes, some with abnormalities of lysosomal biogenesis or melanosome biology. **Griscelli syndrome** patients have silver-gray hair, pigmentary dilution of skin, and melanosomal clumping in hair shafts and the center of melanocytes, with intellectual disability or macrophage activation with hemophagocytosis in different subtypes. All forms are autosomal recessive caused by pathogenic variants in *MLPH, MYO5A,* or *RAB27A*. **Vici syndrome** patients have combined immunodeficiency, intellectual disability, agenesis of the corpus callosum, cataracts, and cleft lip/palate. It is autosomal recessive, caused by pathogenic variants in *EPG5*. Patients with **MAPBP-interacting protein deficiency** have a short stature, recurrent infections, and neutropenia, and inheritance occurs in an autosomal recessive manner due to pathogenic variants in the gene *LAMTOR2*.

Localized Albinism

Localized albinism refers to localized patches of hypopigmentation of skin and hair, which may be evident at birth or develop with time. These conditions are caused by abnormal migration of melanocytes during embryonic development.

Piebaldism

Piebaldism is an autosomal dominant inherited condition in which the individual is usually born with a white forelock. The underlying skin is depigmented and devoid of melanocytes. In addition, there are usually white macules on the face, trunk, and extremities. Pathogenic variants in the *KIT* and *SNAI2* genes have been identified in affected patients.

Waardenburg Syndrome

In Waardenburg syndrome, a white forelock is often associated with lateral displacement of the inner canthi of the eyes, a broad nasal bridge, heterochromia of irides, and sensorineural deafness. This condition is inherited as an autosomal dominant trait with four main subtypes known. Patients with Waardenburg syndrome type 1 (**WS1**, the *most common form*) have all the previous clinical findings, including lateral displacement of the inner canthi. The condition is typically caused by pathogenic variants (>90%) in the *PAX3* gene. Patients with Waardenburg syndrome type 2 **(WS2)** have the clinical findings of WS1 except for the lateral displacement of the inner canthi. Genetically, this is a heterogeneous condition caused by pathogenic variants in several genes, including *MITF, SOX10,* and *SNAI2*. Patients with Waardenburg syndrome type 3 **(WS3)** have all the findings seen in individuals with WS1 plus hypoplasia and contractures of the upper limbs. It is caused by heterozygous or homozygous pathogenic variants of the *PAX3* gene. Finally, Waardenburg syndrome type 4 **(WS4)**, associated with **Hirschsprung disease**, is genetically heterogeneous, with pathogenic variants in different genes (*EDN3, EDNRB,* or *SOX10*) identified.

Other causes of **localized hypopigmentation** include vitiligo and hypomelanosis of Ito (see Chapter 694).

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105.3 Methionine

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The usual pathway for catabolism of methionine, an essential amino acid, produces S-*adenosylmethionine,* which serves as a methyl group donor for methylation reactions for a variety of compounds, and *cysteine,* which is formed through a series of reactions collectively called *trans-sulfuration* (Fig. 105.3).

HOMOCYSTINURIA (HOMOCYSTINEMIA)

Normally, most *homocysteine,* an intermediate compound of methionine degradation, is remethylated to methionine. This methionine-sparing reaction is catalyzed by the enzyme methionine synthase, which requires a metabolite of folic acid (5-methyltetrahydrofolate) as a methyl donor and a metabolite of vitamin B_{12} (methylcobalamin) as a cofactor (see Fig. 105.3). In healthy individuals, most plasma homocysteine is either protein-bound or exists as disulfides. Three major forms of homocystinemia and homocystinuria have been identified.

Homocystinuria Caused by Cystathionine β-Synthase Deficiency (Classic Homocystinuria)

Classic homocystinuria is the most common inborn error of methionine metabolism. It is an autosomal recessive condition caused by biallelic pathogenic variants in *CBS* encoding cystathionine β-synthase. Clinical presentation in the first years of life is nonspecific. Universal newborn screening to detect elevated methionine in dried blood spot samples can identify most affected infants. Second-tier testing after a positive newborn screen result should include plasma methionine and total plasma homocysteine and followed up by confirmatory testing using *CBS* molecular gene analysis. Most affected patients are compound heterozygotes for two different alleles. Heterozygous carriers are asymptomatic.

Infants with classic homocystinuria appear healthy at birth. **Clinical manifestations** during infancy are nonspecific and may include failure to thrive and developmental delay. Without newborn screening, the diagnosis can be delayed and is usually made after 3 years of age, when subluxation of the ocular lens *(ectopia lentis)* occurs. This causes severe myopia and iridodonesis (quivering of the iris). Astigmatism, glaucoma, staphyloma, cataracts, retinal detachment, and optic atrophy may develop later in life. Progressive intellectual disability is common. Neurocognitive scores in the average range have been reported; IQ scores range from 10 to 135. Higher IQ scores are seen in vitamin B_6–responsive patients. Psychiatric and behavioral disorders have been observed in more than 50% of affected patients. Seizures are seen in approximately 20% of untreated patients. Affected individuals with homocystinuria manifest skeletal abnormalities resembling those of Marfan syndrome (see Chapter 743): tall with elongated limbs and arachnodactyly. Scoliosis, pectus excavatum or pectus carinatum, genu valgum, pes cavus, high-arched palate, and crowding of the teeth have been reported. These children usually have a fair complexion, blue eyes, and a peculiar malar flush. Generalized osteoporosis, especially of the spine, is the main x-ray finding. Thromboembolic episodes involving both large and small vessels, especially those of the brain, are common and may occur at any age. Optic atrophy, paralysis, cor pulmonale, and severe hypertension (from renal infarcts) are among the serious consequences of thromboembolism, which is likely caused by elevated homocysteine levels leading to abnormal angiogenesis and inhibition of fibrinolytic activity. The risk of thromboembolism increases after surgical procedures or dehydration. Spontaneous pneumothorax and acute pancreatitis are rare complications.

Elevations of both total plasma homocysteine and methionine in body fluids are the diagnostic laboratory findings. Cysteine is low or absent in plasma. Total plasma homocysteine is the preferred analyte for screening and management of classic homocystinuria. Free plasma homocysteine may normalize or remain normal when total plasma homocysteine is lowered. If urinary homocystine is used for diagnostic purposes, a freshly voided urine sample is preferred because this compound is unstable and may disappear after prolonged storage. The diagnosis may be established by molecular analysis of *CBS.* Prenatal diagnosis is feasible by DNA analysis or by performing an enzyme assay of cultured amniotic cells.

Treatment with high doses of vitamin B_6 (100-200 mg/day) can produce significant improvement in patients who are responsive to this therapy. Approximately 40% of affected patients respond to high doses of vitamin B_6 and usually have milder clinical manifestations than those who are unresponsive to vitamin B_6 therapy. The degree of response to vitamin B_6 treatment may vary across families. Some patients may not respond because of folate depletion; a patient should not be considered unresponsive to vitamin B_6 until folic acid (1-5 mg/day) has been added to the treatment regimen. For patients who are unresponsive to vitamin B_6, lifelong restriction of methionine intake in conjunction with cysteine supplementation is also recommended. The need for dietary restriction and its extent remains controversial in patients with the vitamin B_6–responsive form. In some patients with this form, addition of betaine may obviate the need for any dietary restriction. *Betaine* (trimethylglycine, 6 g/day for adults or 100-200 mg/kg/day for children) lowers homocysteine levels in body fluids by remethylating homocysteine to methionine (see Fig. 105.3), which may result in elevation of plasma methionine levels. This treatment has produced clinical improvement (preventing vascular events) in patients who are unresponsive to vitamin B_6 therapy. Cerebral edema has occurred in a patient with vitamin B_6–nonresponsive homocystinuria and dietary noncompliance during betaine therapy.

More than 100 pregnancies in women with classic homocystinuria have been reported with favorable outcomes for both mothers and infants. The majority of infants were full-term and healthy at birth. Postpartum thromboembolic events occurred in a few mothers.

The screening of newborn infants for classic homocystinuria has been performed worldwide, with an estimated prevalence of 1 in

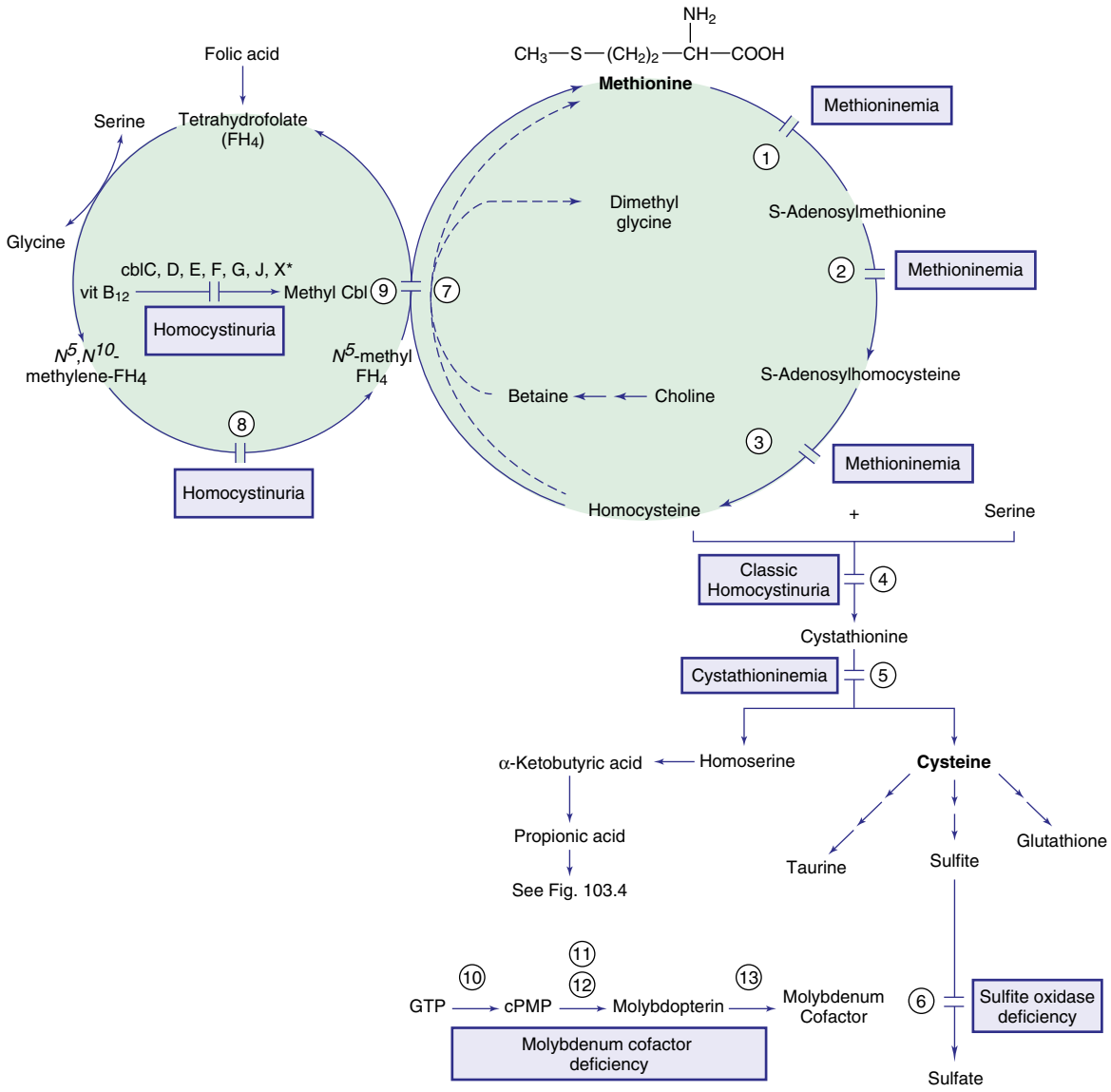

Fig. 105.3 Pathways in the metabolism of sulfur-containing amino acids. **Enzymes:** *(1)* Methionine adenosyltransferase (MAT I/III), *(2)* glycine *N*-methyltransferase, *(3)* *S*-adenosylhomocysteine hydrolase, *(4)* cystathionine synthase, *(5)* cystathionase, *(6)* sulfite oxidase, *(7)* betaine homocysteine methyltransferase, *(8)* methylene tetrahydrofolate reductase, *(9)* methionine synthase (cblG), *(10)* molybdenum cofactor biosynthesis protein 1, *(11)* molybdopterin synthase, *(12)* adenylyltransferase and sulfurtransferase (*MOCS3*), *(13)* gephyrin. GTP, Guanosine triphosphate; cPMP, cyclic pyranopterin monophosphate. *Defects in cblC, D, F, J, and X result in methylmalonic acidemia and homocystinuria.

200,000 to 1 in 350,000 live births, although it can be more common in some parts of the world (e.g., 1 in 1,800 in Qatar). Early treatment of patients identified by screening has produced favorable results. The mean IQ of patients with the vitamin B_6–unresponsive form treated in early infancy was in the normal range. Dislocation of the lens appeared to be preventable in some patients.

Homocystinuria Caused by Defects in Methylcobalamin Formation

Methylcobalamin is the cofactor for methionine synthase, which catalyzes remethylation of homocysteine to methionine. At least seven distinct defects in the intracellular metabolism of cobalamin may interfere with the formation of methylcobalamin (for a more detailed discussion on cobalamin metabolism, see Chapter 105.6 and Figs. 105.3 and 105.4). The seven defects are designated as *cbl*C, *cbl*D (including *cbl*D variant 1), *cbl*E (methionine synthase reductase), *cbl*G (methionine synthase), *cbl*F, *cbl*J, and *cbl*X. Patients with *cbl*C, *cbl*D, *cbl*F, *cbl*J, and *cbl*X defects have **methylmalonic acidemia** *in addition* to homocystinuria, because the formation of both adenosylcobalamin and methylcobalamin is impaired.

Patients with *cbl*E, *cbl*G, and *cbl*D variant one defects are unable to form methylcobalamin and develop homocystinuria without methylmalonic acidemia (Fig. 105.4). The clinical manifestations are similar in patients with these three defects. Nonspecific symptoms such as vomiting, poor feeding, failure to thrive, lethargy, hypotonia, seizures, and developmental delay may occur in the first few months of life. Late-onset forms of these disorders may present with neurocognitive defects, psychosis, and peripheral neuropathy. **Laboratory findings** include megaloblastic anemia, hyperhomocysteinemia, homocystinuria, and hypomethioninemia. The absence of hypermethioninemia differentiates these conditions from cystathionine β-synthase deficiency. Renal artery thrombosis, hemolytic uremic syndrome, pulmonary hypertension, and optic nerve atrophy have been reported in some patients with these defects.

Diagnosis is established by DNA testing or using complementation studies performed in cultured fibroblasts. Prenatal diagnosis can be accomplished by studies in amniotic cell cultures. *cbl*E, *cbl*G, and *cbl*D variant one deficiencies are inherited as autosomal recessive traits. The gene for *cbl*E is *MTRR*, encoding methionine synthase reductase.

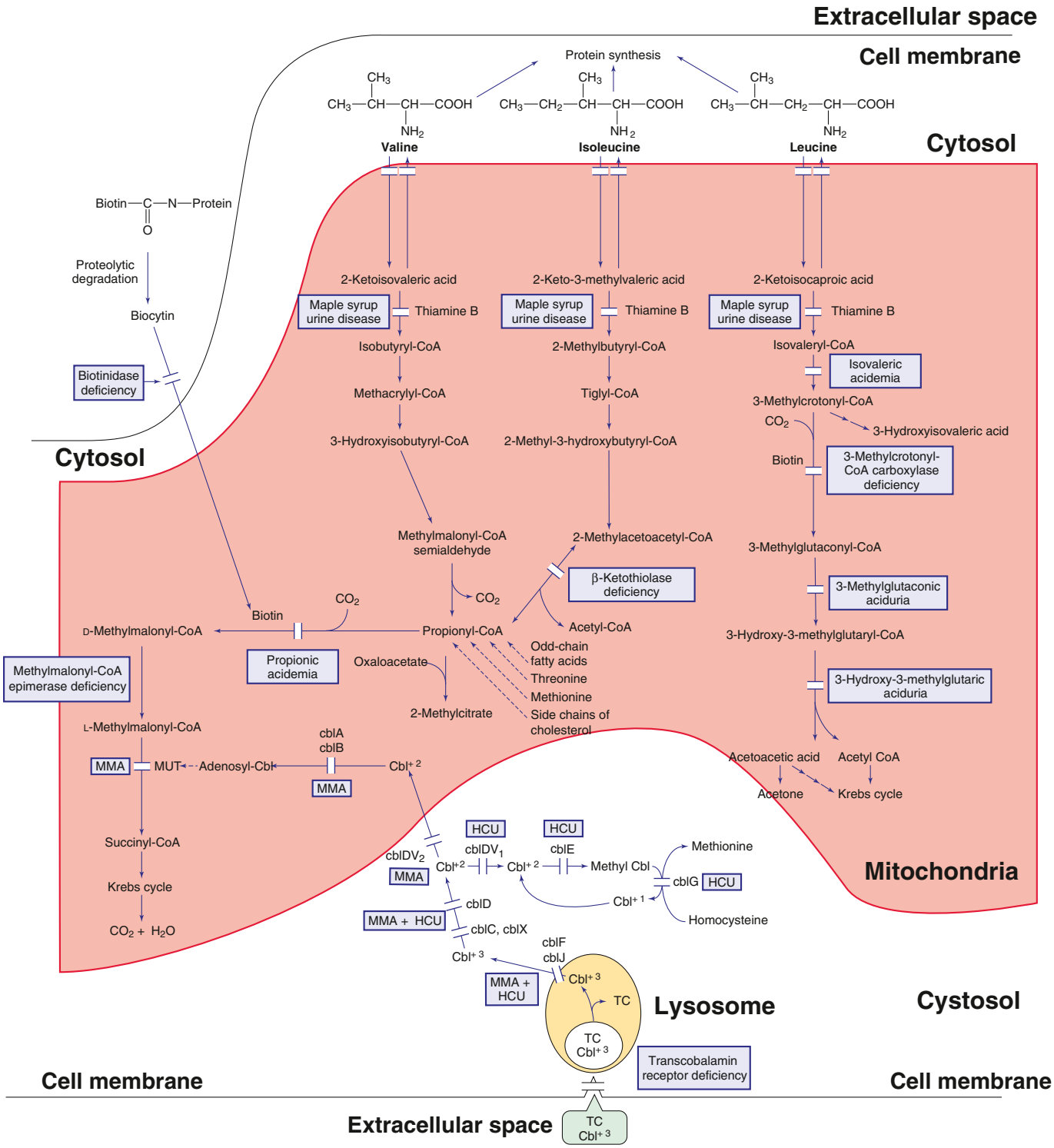

Fig. 105.4 Pathways in the metabolism of the branched-chain amino acids, biotin, and vitamin B_{12} (cobalamin). Adenosyl Cbl, Adenosylcobalamin; Cbl, cobalamin; *cbl*, defect in metabolism of cobalamin; *cbl*DV1, *cbl*D variant 1; *cbl*DV2, *cbl*D variant 2; HCU, homocystinuria; methyl Cbl, methylcobalamin; MMA, methylmalonic acidemia; MUT, mutase; OHCbl, hydroxycobalamin; TC, transcobalamin; TCR, transcobalamin receptor. See text for the name of the enzymes.

The gene for *cbl*G is *MTR*, encoding methionine synthase. The *cbl*D variant one deficiency is caused by pathogenic variants affecting the C-terminal of the *MMADHC* gene.

Treatment with vitamin B_{12} in the form of high-dose parenteral hydroxycobalamin (0.3 mg/kg/day) helps improve the clinical and biochemical findings. Results vary among both diseases and sibships.

Homocystinuria Caused by Deficiency of Methylenetetrahydrofolate Reductase (MTHFR Deficiency)

This enzyme reduces 5,10-methylenetetrahydrofolate to form 5-methyltetrahydrofolate, which provides the methyl group needed for remethylation of homocysteine to methionine (see Fig. 105.3). The severity of the enzyme defect and the clinical manifestations vary considerably

in different families. **Clinical findings** vary from apnea, seizure, microcephaly, coma, and death to developmental delay, ataxia, motor abnormalities, peripheral neuropathy, and psychiatric manifestations. Thromboembolism has also been observed. Exposure to the anesthetic nitrous oxide (which inhibits methionine synthase) in patients with MTHFR deficiency may result in neurologic deterioration and death.

Laboratory findings include moderate hyperhomocysteinemia and homocystinuria. The methionine concentration is low or low-normal. This finding helps differentiate MTHFR deficiency from classic homocystinuria caused by cystathionine β-synthase deficiency. The diagnosis can be confirmed by molecular analysis of *MTHFR* or by the enzyme assay in cultured fibroblasts or leukocytes.

MTHFR deficiency should be differentiated from **mild hyperhomocysteinemia** due to two common variants in the *MTHFR* gene. Two "thermolabile" polymorphisms have been extensively studied: c.665C>T (p.Ala222Val, previously referred to as c.677C>T) and c.1286A>C (p.Glu429Ala, formerly referred to as c.1298A>C). These variants may minimally affect levels of plasma total homocysteine in some patients and are often confounded by dietary folate deficiency. Population-based studies revealed a surprisingly high prevalence of homozygosity for these polymorphisms in the general population: up to 10–15% of North American Whites and >25% in some Hispanics. The best data support a role for the c.665C>T polymorphic variant (formerly c. 677C>T) as a risk factor for neural tube defects. Although clinical tests for this polymorphic variant are widely available, meta-analyses have not supported the association between the *MTHFR* polymorphic variants and risk for venous thromboembolism or between mild hyperhomocysteinemia and an increased risk for coronary heart disease. It is hypothesized that fortification of flour with folate may have decreased the strength of associations observed in the past.

Homocystinuria caused by the loss of function in MTHFR (MTHFR deficiency) is an autosomal recessive disorder. The **diagnosis** can be confirmed by *MTHFR* gene analysis. Prenatal diagnosis can be achieved by molecular analysis of *MTHFR* of known familial pathogenic variants or by measuring MTHFR enzyme activity in cultured chorionic villus cells or amniocytes.

Treatment of MTHFR deficiency with a combination of folic acid, vitamin B_6, vitamin B_{12}, methionine supplementation, and betaine has been tried. Of these, early treatment with betaine appears to have the most beneficial effect.

HYPERMETHIONINEMIA

Primary (Genetic) Hypermethioninemia

Elevation of plasma level of methionine occurs in several genetic conditions. These should be differentiated from acquired (nongenetic) forms of hypermethioninemia, which can occur under various conditions of physiologic stress, especially when involving liver dysfunction.

Classic Homocystinuria

See earlier discussion.

Hepatic Methionine Adenosyltransferase (MAT I/MAT III) Deficiency (Mudd Disease). This hepatic enzyme, which has two isoforms, MAT I (tetrameric) and MAT III (dimeric), is encoded by a single gene, *MAT1A,* and is involved in the first step of methionine catabolism (see Fig. 105.3). Another structurally similar enzyme, MAT II, is encoded by a different gene, *MAT2A,* and is expressed predominantly in nonhepatic tissues (e.g., kidney, brain, lymphocytes). Deficiency of MAT I/MAT III causes hypermethioninemia. In severe deficiency, total plasma homocysteine can also be elevated. The majority of these patients have been diagnosed in the neonatal period through screening for classic homocystinuria. Most affected individuals have residual enzyme activity and remain asymptomatic throughout life despite persistent hypermethioninemia. Some complain of an unusual odor to their breath, likely caused by accumulation of dimethylsulfide. A few patients with complete enzyme deficiency have had neurologic abnormalities related to demyelination (intellectual disability, dystonia, dyspraxia).

Laboratory studies reveal markedly elevated levels of plasma methionine with a normal or low level of *S*-adenosylmethionine, normal concentrations of *S*-adenosylhomocysteine, and normal or slightly elevated total plasma homocysteine. These findings help differentiate MAT I/MAT III deficiency from other causes of hypermethioninemia.

No uniformly accepted therapeutic regimen has yet emerged. Long-term follow-up to monitor for neurologic and liver abnormalities should be considered. Diets low in methionine result in lowering of plasma methionine, but the advisability of such diets has been questioned because lowering plasma methionine, an essential amino acid, causes further lowering of *S*-adenosylmethionine, thus interfering with reactions of remethylation. Supplementation with *S*-adenosylmethionine in conjunction with a low-methionine diet seems prudent, but no large clinical experience is yet available. Normal pregnancies producing normal offspring have been reported in mothers with MAT I/MAT III *(MAT1A)* deficiency. The condition is inherited as an autosomal recessive trait, although the pathogenic variant p.Arg264His in *MAT1A* appears to disrupt protein dimerization and may result in mild hypermethioninemia even in heterozygous patients.

Glycine *N*-Methyltransferase Deficiency. Glycine *N*-methyltransferase *(GNMT)* mediates catabolism of *S*-adenosylmethionine to *S*-adenosylhomocysteine (see Fig. 105.3). A few patients with deficiency of this enzyme have been reported to date. Clinically, patients were asymptomatic except for mild hepatomegaly and elevated serum levels of aminotransferases. Other laboratory findings included hypermethioninemia and very high levels of serum *S*-adenosylmethionine. No specific treatment has yet been identified. The condition is inherited as an autosomal recessive trait.

S-Adenosylhomocysteine Hydrolase (SAHH) Deficiency. SAHH deficiency (see Fig. 105.3) has been described infrequently. Intellectual disability, severe hypotonia, and progressive liver dysfunction were common clinical findings. Laboratory studies included elevated levels of serum creatine kinase, hypoalbuminemia (associated with fetal hydrops in one family), hypoprothrombinemia, and greatly elevated levels of serum *S*-adenosylhomocysteine with moderate elevations of plasma methionine and *S*-adenosylmethionine. Marked elevation in *S*-adenosylhomocysteine has been thought to cause inhibition of methyltransferases, including those involved in the synthesis of creatine (see Fig. 105.10) and choline, resulting in their deficiencies. Brain MRIs often reveal delayed myelination of the white matter. The diagnosis can be achieved by *AHCY* gene analysis or, if needed, by biochemical assay of red blood cells, cultured skin fibroblasts, or liver biopsy. Treatment with a low-methionine diet has been used, but its long-term effectiveness has not been established.

Tyrosinemia Type I. See Chapter 105.2.

Citrin Deficiency. See Chapter 105.12.

Acquired (Nongenetic) Hypermethioninemia

Hypermethioninemia occurs in premature and some full-term infants receiving high-protein diets, in whom it may represent delayed maturation of the enzyme MAT. Adjusting protein intake under the control of plasma methionine usually resolves the abnormality. Hypermethioninemia is also commonly found in patients with various forms of liver disease.

PRIMARY CYSTATHIONINEMIA (CYSTATHIONINURIA)

Cystathionase (cystathionine γ-lyase encoded by *CTH*) deficiency results in massive cystathioninuria and mild to moderate cystathioninemia. Cystathionase deficiency of this enzyme is inherited as an autosomal recessive trait, with an estimated prevalence of 1 in 14,000 live births. A wide variety of clinical manifestations have been reported. Lack of a consistent clinical picture and the presence of cystathioninuria in some individuals *without* apparent clinical findings suggest that cystathionase deficiency may be of no clinical significance. Many reported cases are responsive to oral administration of large doses of vitamin B_6 (~100 mg/day). When cystathioninuria is discovered in a patient, vitamin B_6 treatment can be tried, but its beneficial effect has not been established.

Primary cystathioninuria needs to be differentiated from secondary cystathioninuria, which can occur in patients with vitamin B_6 and/or

B_{12} deficiency, liver disease (particularly damage caused by galactosemia), thyrotoxicosis, hepatoblastoma, neuroblastoma, ganglioblastoma, or defects in remethylation of homocysteine.

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105.4 Cysteine and Cystine

Oleg A. Shchelochkov and Charles P. Venditti

Cysteine is a sulfur-containing amino acid synthesized from methionine (see Fig. 105.3). Oxidation of cysteine forms *cystine,* a poorly soluble dimer. The most common genetic disorders of cysteine and cystine metabolism are cystinuria (see Chapter 584) and cystinosis (see Chapter 569.3).

SULFITE OXIDASE DEFICIENCY AND MOLYBDENUM COFACTOR DEFICIENCY

In the last step in cysteine metabolism, sulfite is oxidized to sulfate by sulfite oxidase, and the sulfate is excreted in the urine (see Fig. 105.3). Sulfite oxidase is encoded by *SUOX* and requires a molybdenum-pterin complex called *molybdenum cofactor.* This cofactor is also necessary for the function of two other enzymes in humans: xanthine dehydrogenase (oxidizes xanthine and hypoxanthine to uric acid) and aldehyde oxidase (involved in oxidizing a number of natural compounds and drugs). Three enzymes, encoded by three different genes (*MOCS1, MOCS2,* and *GPHN*), are involved in the synthesis of the cofactor. Deficiency of any of the three enzymes causes cofactor deficiency with similar phenotypes. Most patients, who were originally diagnosed as having **sulfite oxidase deficiency**, have been shown to have **molybdenum cofactor deficiency**. Sulfite oxidase deficiency and molybdenum cofactor deficiency are autosomal recessive disorders.

The enzyme and cofactor deficiencies present with overlapping and often nonspecific **clinical manifestations**. Refusal to feed, vomiting, an exaggerated startle reaction, severe intractable seizures (tonic, clonic, myoclonic), cortical atrophy with subcortical multicystic lesions, and severe developmental delay may develop within a few weeks after birth. The biochemical diagnosis should be considered in infants presenting with neonatal seizures and neonates with symptoms reminiscent of hypoxic-ischemic encephalopathy. Bilateral dislocation of ocular lenses is a common finding in patients who survive the neonatal period. The intractable seizures seen in this condition are in part a consequence of secondary vitamin B_6 dependency. The accumulation of sulfites in body fluids in this condition causes the inhibition of the *antiquitin* enzyme, which is necessary for the conversion of α-amino adipic semialdehyde to α-aminoadipic acid; the resultant accumulation of α-aminoadipic semialdehyde and its cyclic form, P6C, causes the inactivation of pyridoxal-5-phosphate (the active form of vitamin B_6), leading to **vitamin B_6–dependent** epilepsy (see also Chapter 105.14).

Affected children excrete large amounts of sulfite, thiosulfate, *S*-sulfocysteine, xanthine, and hypoxanthine in the urine. Serum uric acid and urinary excretion of sulfate and uric acid are diminished. Fresh urine should be used for screening purposes and for quantitative measurements of sulfite, because oxidation of sulfite to sulfate at room temperature may produce false-negative results. Increased concentrations of α-aminoadipic semialdehyde and P6C can be found in the CSF, plasma, and urine.

Diagnosis is confirmed by analysis of affected genes (often using a multigene panel). Infrequently, measurement of sulfite oxidase and molybdenum cofactor in fibroblasts and liver biopsies is required. Prenatal diagnosis is possible by DNA studies or by performing an assay of sulfite oxidase activity in cultured amniotic cells, in samples of chorionic villi. The prevalence of these deficiencies in the general population is not known but likely is very low.

Patients with *MOCS1*-related molybdenum cofactor deficiency may benefit from daily intravenous **cyclic pyranopterin monophosphate** (a cPMP analogue), a compound under investigation in a multicenter clinical trial. Large doses of vitamin B_6 may alleviate the frequency and severity of seizures but do not seem to alter the devastating neurologic outcome. A cysteine-restricted diet under control of plasma amino acids to avoid essential amino acid deficiencies can be attempted in some patients, but the long-term efficacy of this approach is unknown.

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105.5 Tryptophan

Oleg A. Shchelochkov and Charles P. Venditti

Tryptophan is an essential amino acid and a precursor for nicotinic acid (niacin) and serotonin (Fig. 105.5). The genetic disorders of metabolism of serotonin, one of the major neurotransmitters, are discussed in Chapter 105.11.

HARTNUP DISEASE

Hartnup disease is an autosomal recessive condition caused by biallelic pathogenic variants in *SLC6A19*. This gene encodes a monoamino-monocarboxylic amino acid transporter (B^0AT1), which facilitates transport of neutral amino acids, including tryptophan, across the intestinal mucosa and renal tubules. Patients show significant variability in presentation, likely related to nutritional factors, environment, and genetic heterogeneity of modifier genes (e.g., two proteins, TMEM27 and ACE2, that interact with B^0AT1). The prevalence of Hartnup disorder is estimated to be 1 in 20,000 to 1 in 55,000 live births. Most children with Hartnup disease remain asymptomatic. Decreased intestinal absorption of tryptophan in conjunction with its increased renal loss can lead to reduced availability of tryptophan for niacin synthesis. **Tryptophan deficiency** can be accentuated by malabsorption such as celiac disease. One of the more noticeable clinical manifestations in the rare symptomatic patient is **cutaneous photosensitivity**. The skin becomes rough and red after moderate exposure to the sun, and with greater exposure, a pellagra-like rash may develop. The rash may be pruritic, and a chronic eczema may develop. The skin changes have been reported in affected infants as young as 10 days of age. Some patients may have intermittent ataxia manifested as an unsteady, wide-based gait. The ataxia may last a few days and can respond to niacin supplementation. Cognitive development is usually normal. Episodic psychiatric manifestations such as irritability, emotional instability, depression, and suicidal tendencies, have been observed; these changes are usually associated with bouts of ataxia. Short stature and atrophic glossitis are seen in some patients.

Most children diagnosed with Hartnup disorder by neonatal screening have remained asymptomatic. This indicates that other factors are also involved in the pathogenesis of the clinical condition.

The main laboratory finding is **aminoaciduria**, which is restricted to neutral amino acids (alanine, serine, threonine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, histidine). Urinary excretion of proline, hydroxyproline, and arginine remains normal. This finding helps differentiate Hartnup disorder from other causes of generalized aminoaciduria, such as renal Fanconi syndrome. Plasma concentrations of neutral amino acids are normal or mildly decreased. This seemingly unexpected finding reflects compensatory mechanisms that maintain normal transport and utilization of amino acids. The indole derivatives (especially indican) may be found in large amounts in some patients, resulting from bacterial breakdown of unabsorbed tryptophan in the intestines.

Diagnosis of Hartnup disorder is established by the intermittent nature of symptoms and characteristic findings on the urine amino acid analysis. When necessary, the diagnosis can be confirmed molecularly by *SLC6A19* gene analysis.

Treatment with nicotinic acid or nicotinamide (50-300 mg/day) and a high-protein diet results in a favorable response in symptomatic patients with Hartnup disorder. Because of the intermittent nature of the clinical manifestations, the efficacy of these treatments is difficult to establish. Normal outcome for both the mother and fetus has been reported in several affected women.

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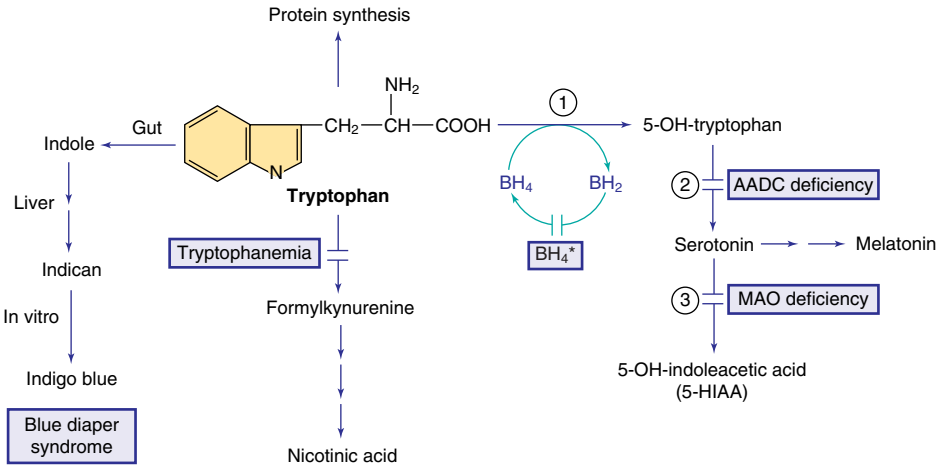

Fig. 105.5 Pathways in the metabolism of tryptophan. BH_4* indicates hyperphenylalaninemia caused by tetrahydrobiopterin deficiency (see Fig. 105.1). **Enzymes:** *(1)* Tryptophan hydroxylase, *(2)* aromatic L-amino acid decarboxylase (AADC), *(3)* monoamine oxidase (MAO).

105.6 Isoleucine, Leucine, Valine, and Related Organic Acidemias

Oleg A. Shchelochkov, Irini Manoli, and Charles P. Venditti

The early steps in the degradation of the branched-chain amino acids (BCAAs)—isoleucine, leucine, and valine—are similar (see Fig. 105.4). Under catabolic conditions, BCAAs in the muscle tissue undergo a reversible reaction of transamination catalyzed by BCAA transaminase. α-Ketoacids formed by this reaction then undergo an oxidative decarboxylation step mediated by a branched-chain α-ketoacid dehydrogenase **(BCKDH)** complex. The deficiency of BCKDH results in **maple syrup urine disease**, whereas the deficiency of enzymes mediating more distal reactions results in accumulation of enzyme-specific levels of organic acids excreted in the urine, thus giving those inborn errors of metabolism the eponyms **organic acidemias** and **organic acidurias**. These disorders typically cause metabolic acidosis, which usually occurs in the first few days of life. Although most of the clinical findings are nonspecific, some manifestations may provide important clues to the nature of the enzyme deficiency. Figure 105.6 presents an approach to infants suspected of having an organic acidemia. The diagnosis is usually established by identifying and measuring specific organic acids in body fluids (blood, urine), amino acid analysis (blood), and identifying pathogenic variants in the respective gene.

Organic acidemias *are not limited* to defects in the catabolic pathways of BCAAs. Disorders causing accumulation of other organic acids include those derived from lysine (see Chapter 105.14), disorders of the γ-glutamyl cycle (see Chapter 105.11), those associated with lactic acid (see Chapter 107), and dicarboxylic acidemias associated with defective fatty acid degradation (see Chapter 106).

MAPLE SYRUP URINE DISEASE

Decarboxylation of leucine, isoleucine, and valine is accomplished by a complex enzyme system (BCKDH) using thiamine (vitamin B_1) pyrophosphate as a coenzyme. This mitochondrial enzyme consists of four subunits: $E_{1\alpha}$, $E_{1\beta}$, E_2, and E_3. The E_3 subunit is shared with two other dehydrogenases: pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. Deficiency of any of these subunits causes maple syrup urine disease **(MSUD)** (see Fig. 105.4), a disorder named after the sweet odor of maple syrup discerned from body fluids, especially cerumen, sweat, and urine. Clinical conditions caused by defects in $E_{1\alpha}$, $E_{1\beta}$, E_2 and E_3 are designated as MSUD type 1A, type 1B, type 2, and type 3, respectively. This classification, however, is not very helpful clinically because the severity of clinical manifestations does not correlate with, or correspond specifically to, any single enzyme subunit. An affected infant with a type 1A defect can have clinical manifestations ranging from relatively mild to very severe. A more useful classification, based on clinical findings and response to thiamine administration, delineates five phenotypes of MSUD.

Classic Maple Syrup Urine Disease

Classic MSUD has the most severe clinical manifestations. The BCKDH complex activity in this group varies between 0% and 2% of controls. Patients with uncontrolled or poorly controlled disease develop signs of acute encephalopathy. The mechanisms underlying this life-threatening complication are complex, but leucine and its derivative, α-ketoisocaproic acid, appear to be the key factors underlying acute encephalopathy. Elevated leucine competitively inhibits the uptake of other amino acids by the LNAA transporter. Once taken up by the brain tissue, leucine is metabolized by BCAA aminotransferase to α-ketoisocaproic acid, which disrupts metabolism of neurotransmitters and amino acids (glutamate, γ-aminobutyric acid [GABA], glutamine, alanine, and aspartate). α-Ketoisocaproic acid can reversibly inhibit oxidative phosphorylation and result in cerebral lactic acidosis. Collectively, these processes are detrimental to the normal function of neurons and glia, clinically manifesting as *encephalopathy* and *brain edema,* referred to as **leucinosis**. Affected infants who appear healthy at birth develop poor feeding and vomiting in the first days of life. Lethargy and coma may ensue within a few days. Physical examination reveals hypertonicity and muscular rigidity with severe opisthotonos. Periods of hypertonicity may alternate with bouts of flaccidity manifested as repetitive movements of the extremities ("boxing" and "bicycling"). Neurologic findings are often mistakenly thought to be caused by generalized sepsis and meningitis. Cerebral edema may be present; seizures occur in most infants, and hypoglycemia is common. In contrast to most hypoglycemic states, correction of the blood glucose concentration *does not* improve the clinical condition. Aside from the blood glucose and varying degrees of ketoacidosis, routine laboratory findings are usually unremarkable. If left untreated, death can occur in the first few weeks or months of life.

Diagnosis was classically suspected because of the peculiar odor of maple syrup in urine, sweat, and cerumen. It is usually confirmed by amino acid analysis showing marked elevations in plasma levels of leucine, isoleucine, valine, and alloisoleucine and a depressed level of alanine. Alloisoleucine is a stereoisomer of isoleucine not normally found in blood and is the most sensitive and specific diagnostic marker for all forms of MSUD. Leucine levels are usually higher than those of isoleucine and valine. Urine contains high levels of leucine, isoleucine, and valine and their respective ketoacids. These ketoacids may be detected qualitatively by adding a few drops of 2,4-dinitrophenylhydrazine reagent (0.1% in 0.1N HCl) to the urine; a yellow precipitate of 2,4-dinitrophenylhydrazone is formed in a positive test. Neuroimaging during the acute state may show cerebral edema, which is most

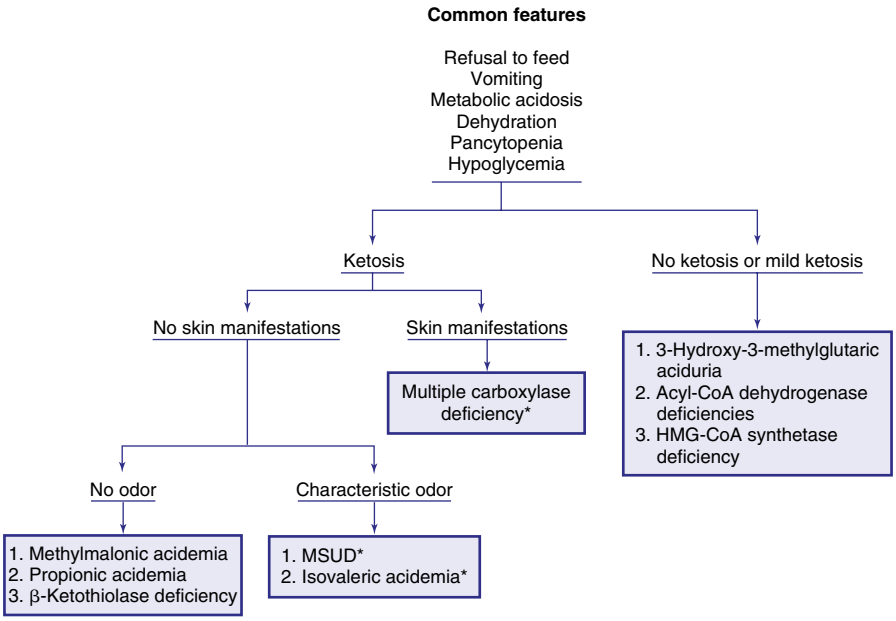

Fig. 105.6 Clinical algorithmic approach to infants with organic acidemia. *Asterisks* indicate disorders in which patients have a characteristic odor (see text and Table 105.2). MSUD, Maple syrup urine disease.

prominent in the cerebellum, dorsal brainstem, cerebral peduncle, and internal capsule. After recovery from the acute state and with advancing age, hypomyelination and cerebral atrophy may be seen in neuroimaging of the brain.

Treatment of the acute state is aimed at hydration and rapid removal of the BCAAs and their metabolites from the tissues and body fluids. Uptake of leucine by the brain and accumulation of the downstream metabolite, α-ketoisocaproic acid, appear to be the key metabolic events underlying the MSUD encephalopathy. Therefore MSUD management strategies focus on decreasing plasma leucine to control acute and chronic manifestations of the disease.

Because renal clearance of leucine is low, hydration alone may not produce a rapid improvement. *Hemodialysis* is the most effective mode of therapy in critically ill infants and should be instituted promptly; significant decreases in plasma levels of leucine, isoleucine, and valine are usually seen within 24 hours. Sufficient calories and nutrients should be provided intravenously or orally as soon as possible to reverse the patient's catabolic state. Cerebral edema, if present, may require treatment with mannitol, diuretics (e.g., furosemide), or hypertonic saline. Counterintuitively, supplementation with isoleucine and valine is also needed to achieve adequate control of the plasma leucine level in MSUD patients and to sustain the net protein synthesis in tissues. Judiciously administered isoleucine and valine will compete with leucine for the LNAA transporter at the BBB, decrease leucine entry into the CNS, and help in the prevention and treatment of leucine encephalopathy. Phenylbutyrate, a nitrogen scavenger used to treat hyperammonemia in urea cycle disorders, has been shown to activate the BCKDH complex and lower BCAA levels, with promising results in a subset of MSUD patients.

Treatment after recovery from the acute state requires a diet low in BCAAs. Synthetic formulas devoid of leucine, isoleucine, and valine are available commercially. Because these amino acids cannot be synthesized endogenously, age-appropriate amounts of BCAAs should be provided in the diet in the form of complete protein. To avoid essential amino acid deficiencies, the amount should be titrated carefully by performing frequent analyses of plasma amino acids, with close attention to plasma isoleucine, leucine, and valine levels. A clinical condition resembling **acrodermatitis enteropathica** (see Chapter 691) occurs in affected infants whose plasma isoleucine or valine had been *overrestricted*; the addition of isoleucine or valine, respectively, to the diet will hasten the recovery of the skin rash. Patients with MSUD need to remain on the diet for life. Liver transplantation is offered to patients with classic MSUD, allowing some patients to lift protein restrictions and improve metabolic control during intercurrent illnesses.

The long-term prognosis of affected children remains guarded. Severe ketoacidosis, cerebral edema, and death may occur during any stressful situation such as infection or surgery, especially in mid-childhood. Cognitive and other neurologic deficits are common sequelae.

Intermediate (Mild) Maple Syrup Urine Disease

Children with intermediate MSUD develop milder disease after the neonatal period. Clinical manifestations are insidious and limited to the CNS. Patients have mild to moderate intellectual disability with or without seizures. They have the odor of maple syrup and excrete moderate amounts of the BCAAs and their ketoacid derivatives in the urine. Plasma concentrations of leucine, isoleucine, and valine are moderately increased, whereas those of lactate and pyruvate tend to be normal. These children are commonly diagnosed during an intercurrent illness, when signs and symptoms of classic MSUD may occur. The dehydrogenase activity is 3–40% of the reference population. Because patients with thiamine-responsive MSUD usually have manifestations similar to the mild form, a trial of thiamine therapy is recommended. Diet therapy, similar to that of classic MSUD, is needed.

Intermittent Maple Syrup Urine Disease

In intermittent MSUD, seemingly normal children develop vomiting, odor of maple syrup, ataxia, lethargy, and coma during any stress or catabolic state such as infection or surgery. During these attacks, laboratory findings are indistinguishable from those of the classic form, and death may occur. Treatment of the acute attack of intermittent MSUD is similar to that of the classic form. After recovery, although a normal diet can be tolerated, a low-BCAA diet is recommended. The BCKDH activity in patients with the intermittent form is higher than in the classic form and may reach 40% of the control activity.

Thiamine-Responsive Maple Syrup Urine Disease

Some children with mild or intermediate forms of MSUD who are treated with high doses of thiamine show dramatic clinical and biochemical improvement. Although some respond to treatment with thiamine at 10 mg/day, others may require as much as 100 mg/day for at least 3 weeks before a favorable response is observed. These patients also require a BCAA-restricted diet. The enzymatic activity in these patients can be up to 40% of the reference population.

Maple Syrup Urine Disease Caused by Deficiency of E_3 Subunit (MSUD Type 3)

Although sometimes referred to as "maple syrup urine disease type 3," this very rare disorder leads to clinical and biochemical abnormalities that encompass a wide range of mitochondrial reactions. The E_3 subunit, dihydrolipoamide dehydrogenase, is a component of the BCKDH complex, pyruvate dehydrogenase complex, and α-ketoglutarate dehydrogenase complex. Causative biallelic pathogenic variants in *DLD* that encode the E_3 dihydrolipoamide dehydrogenase cause lactic acidosis, elevated pyruvate, and signs and symptoms similar to intermediate MSUD. Progressive neurologic impairment manifested by hypotonia and developmental delay occurs after 2 months of age. Abnormal movements can progress to ataxia or Leigh syndrome. Death may occur in early childhood.

Laboratory findings include persistent lactic acidosis with high levels of plasma pyruvate and alanine. Plasma BCAA concentrations are moderately increased. Patients excrete large amounts of lactate, pyruvate, α-ketoglutarate, and the three branched-chain ketoacids in their urine.

No effective treatment is available. BCAA-restricted diets and treatment with high doses of thiamine, biotin, and lipoic acid have been ineffective.

Genetics and the Prevalence of Maple Syrup Urine Disease

Biallelic pathogenic variants in any of the following genes may result in the clinical and biochemical manifestations of MSUD: *BCKDHA* (encodes subunit $E_{1\alpha}$), *BCKDHB* (encodes subunit $E_{1\beta}$), *DBT* (encodes subunit E_2), and *DLD* (encodes subunit E_3). Genotype-phenotype correlations are difficult to establish and are usually imprecise. The exception is thiamine-responsive MSUD, shown to be caused by pathogenic variants in *DBT*. Most patients are compound heterozygotes inheriting two different pathogenic alleles. Pathogenic variants in *BCKDHA* (45%) and *BCKDHB* (35%) account for approximately 80% of cases. Pathogenic variants in *DBT* are responsible for 20% of MSUD cases.

The prevalence is estimated at 1 in 185,000 live births. Classic MSUD is more prevalent in the Old Order Mennonites in the United States, at an estimated 1 in 380 live births. Affected patients in this population are homozygous for a specific pathogenic variant (c.1312T>A) in the *BCKDHA*-encoding $E_{1\alpha}$ subunit.

Early detection of MSUD is possible by universal newborn screening. In many cases, however, especially those with classic MSUD, the infant may be very ill by the time screening results become available (see Chapter 104). Prenatal diagnosis has been accomplished by enzyme assay of the cultured amniocytes, cultured chorionic villus tissue, or direct assay of samples of the chorionic villi and by identification of the known pathogenic variants in the affected gene.

Successful pregnancies have occurred in women with different forms of MSUD. The teratogenic potential of leucine during pregnancy is unknown. Tight control of isoleucine, leucine, and valine before and during the pregnancy is important to minimize the risk of metabolic decompensation and to optimize fetal nutrition. Mothers affected by MSUD require close monitoring and meticulous management of nutrition, electrolytes, and fluids in the postpartum period.

BRANCHED-CHAIN α-KETOACID DEHYDROGENASE KINASE DEFICIENCY

A defect in the regulation of BCKDH by BCKDH kinase (BCKDK), the enzyme responsible for phosphorylation-mediated inactivation of the BCKDH complex, causes the *reverse* biochemical phenotype of MSUD. Pathogenic variants in *BCKDK* decrease the negative regulation by the kinase, resulting in uncontrolled degradation and depletion of isoleucine, leucine, and valine in plasma and the brain. Patients with BCKDK deficiency present with low plasma concentrations of isoleucine, leucine, and valine associated with autism, intellectual impairment, fine motor coordination problems, and seizures.

BRANCHED-CHAIN AMINO ACID TRANSPORTER DEFICIENCY

Isoleucine, leucine, and valine are transported across the BBB mainly by the heterodimeric LNAA transporter LAT1 encoded by *SLC7A5*. A defect in LAT1 caused by pathogenic variants in *SLC7A5* results in low brain concentrations of isoleucine, leucine, and valine. Patients with this defect may present similarly to BCKDK-deficient patients, with autism, microcephaly, gross motor delays, and in some cases, seizures.

ISOVALERIC ACIDEMIA

Isovaleric acidemia **(IVA)** is an autosomal recessive condition caused by biallelic pathogenic variants in *IVD* resulting in the deficient activity of isovaleryl–coenzyme A (CoA) dehydrogenase (see Fig. 105.4). The prevalence of IVA is estimated to range between 1 in 62,500 (in parts of Germany) and 1 in 250,000 live births (in the United States). Decreased or absent activity of isovaleryl-CoA dehydrogenase results in impaired leucine degradation. Accumulating derivatives of isovaleric acid, isovalerylcarnitine, isovalerylglycine, and 3-hydroxyisovaleric acid can be detected in body fluids and thus enable the biochemical diagnosis and screening. Clinically, the course of IVA can be highly variable, ranging from essentially asymptomatic to severe. Introduction of newborn screening and proactive management of IVA has changed the prognosis and clinical course. Older siblings of symptomatic newborn infants have been reported with identical genotype and biochemical abnormalities but without clinical manifestations, suggesting that presymptomatic detection of affected patients on the newborn screen can improve clinical outcomes.

Patients with severe IVA can present with vomiting, severe acidosis, hyperammonemia, hypoglycemia, hypocalcemia, and bone marrow suppression in infancy. Lethargy, convulsions, and coma may ensue, and death may occur if proper therapy is not initiated. Vomiting may be severe enough to suggest pyloric stenosis. The characteristic odor of "sweaty feet" or "rancid cheese" may be present. Infants who survive this acute episode are at risk of developing episodes of metabolic decompensation later in life. In the mild form without treatment, typical clinical manifestations of severe IVA (vomiting, lethargy, acidosis, or coma) may not appear until the child is a few months or years old. Acute episodes of metabolic decompensation may occur during a catabolic state, such as infection, dehydration, surgery, or high-protein intake. Acute episodes may be mistaken for diabetic ketoacidosis. Some patients may experience acute and recurrent episodes of pancreatitis.

Laboratory findings during the acute attacks include ketoacidosis, neutropenia, thrombocytopenia, and occasionally pancytopenia. Hypocalcemia, hypoglycemia, and moderate to severe hyperammonemia may be present in some patients. Increases in plasma ammonia may suggest a defect in the urea cycle (see Chapter 105.12). In urea cycle defects, the infant usually shows no significant ketoacidosis (see Fig. 105.6).

Diagnosis is established by demonstrating marked elevations of isovaleric acid metabolites (isovalerylglycine, 3-hydroxyisovaleric acid) in body fluids, especially urine. The main compound in plasma is isovalerylcarnitine (C5-carnitine). C5-carnitine can be measured in dried blood spots, enabling universal newborn screening using tandem mass spectrometry. The diagnosis can be confirmed by molecular analysis of the *IVD* gene. In some patients with equivocal results, measurement of the enzyme activity in cultured skin fibroblasts may be necessary.

Treatment of the acute attack is aimed at rehydration, reversal of the catabolic state (by providing adequate calories orally or intravenously), correction of metabolic acidosis, and facilitation of the isovaleric acid excretion. L-Carnitine (100 mg/kg/day orally) also increases removal of isovaleric acid by forming isovalerylcarnitine, which is excreted in the urine. Because isovalerylglycine has a high urinary clearance, some centers recommend glycine supplementation (250 mg/kg/day) to enhance the formation of isovalerylglycine. Temporary restriction of protein intake (<24 hours) may be beneficial in some cases. In patients with significant hyperammonemia (blood ammonia >200 μmol/L), measures that reduce blood ammonia should be employed (see Chapter 105.12). Renal replacement therapy may be needed if the previously described measures fail to produce significant clinical and biochemical improvement. Long-term management of IVA patients requires restriction of protein according to age-appropriate intake (recommended dietary allowance of protein). Patients benefit from carnitine supplementation with or without glycine. Normal development can be achieved with early and proper treatment.

Prenatal diagnosis can be accomplished using *IVD* gene analysis if causative pathogenic variants are known or, less frequently, by using an enzyme assay of cultured amniocytes. Successful pregnancies with favorable outcomes have been reported. Universal newborn screening of IVA is used in the United States and other countries (see Chapter 104).

MULTIPLE CARBOXYLASE DEFICIENCIES (DEFECTS OF THE BIOTIN CYCLE)

Biotin is a water-soluble vitamin that serves as a cofactor for four carboxylase enzymes in humans: pyruvate carboxylase, acetyl-CoA carboxylase, propionyl-CoA carboxylase, and 3-methylcrotonyl-CoA carboxylase. The latter two are involved in the catabolic pathways of leucine, isoleucine, and valine (see Fig. 105.4).

Most dietary biotin is bound to proteins. Free biotin is generated in the intestine by the action of digestive enzymes, by intestinal bacteria, and perhaps by biotinidase. **Biotinidase**, found in serum and most tissues, is also essential for the recycling of biotin in the body by releasing it from the apoenzymes (carboxylases; see Fig. 105.4). Free biotin must form a covalent bond with the apocarboxylases to produce the activated enzyme (holocarboxylase). This binding is catalyzed by holocarboxylase synthetase. Deficiencies either in holocarboxylase synthetase or in biotinidase result in the impaired catalytic activity of carboxylases and organic acidemias.

Holocarboxylase Synthetase Deficiency

Infants with this rare autosomal recessive disorder become symptomatic in the first few weeks of life. Symptoms may appear as early as a few hours after birth to as late as 8 years of age. Clinically, shortly after birth, affected infants develop breathing difficulties (tachypnea and apnea). Feeding problems, vomiting, and hypotonia are also usually present. If the condition remains untreated, *generalized erythematous rash with exfoliation and alopecia,* failure to thrive, irritability, seizures, lethargy, and even coma may occur. Developmental delay is common. Immune deficiency manifests with susceptibility to infection. Urine may have a peculiar odor, which has been described "tomcat urine." The rash, when present, helps to differentiate this condition from other organic acidemias (see Fig. 105.6).

Laboratory findings include metabolic acidosis, ketosis, hyperammonemia, and the presence of a variety of organic acids and their conjugates (lactic acid, 3-methylcrotonic acid, 3-methylcrotonylglycine, tiglylglycine, 3-OH-propionic acid, methylcitric acid, and 3-hydroxyisovaleric acid) in body fluids. **Diagnosis** is confirmed by identification of pathogenic variants in *HLCS* or by the enzyme assay in lymphocytes or cultured fibroblasts. Most pathogenic variants cause the enzyme to have an increased K_m (Michaelis-Menten dissociation constant) for biotin; the enzyme activity in such patients can be restored by the administration of large doses of biotin. Newborn screening can identify holocarboxylase synthetase–deficient infants by detecting elevated C5-OH-carnitine on tandem mass spectrometry. In these infants, biotinidase enzymatic assay would be normal.

Treatment with biotin (10-20 mg/day orally) usually results in an improvement in clinical manifestations and biochemical abnormalities. Early diagnosis and treatment are critical to prevent irreversible neurologic damage. In some patients, complete resolution may not be achieved even with large doses (up to 60 mg/day) of biotin.

Prenatal diagnosis can be accomplished by prenatal molecular analysis of known pathogenic variants in *HLCS* or by assaying enzyme activity in cultured amniotic cells. Pregnant mothers who had previous offspring with holocarboxylase synthetase deficiency have been treated with biotin late in pregnancy. Affected infants were normal at birth, but the efficacy of prenatal treatment remains unclear.

Biotinidase Deficiency

Impaired biotinidase activity results in biotin deficiency. Affected infants may develop clinical manifestations similar to those seen in infants with holocarboxylase synthetase deficiency. Unlike the latter, however, symptoms tend to appear later, when the child is several months or years old. The delay in onset of symptoms presumably results from the availability of free biotin derived from the mother or the diet. Clinical manifestations are mostly confined to the skin and the nervous system. Without treatment, atopic or seborrheic dermatitis, candidiasis, alopecia, ataxia, seizures (usually myoclonic), hypotonia, developmental delay, optic nerve atrophy, sensorineural hearing loss, and immunodeficiency resulting from impaired T-cell function may occur. A small number of children with *intractable seborrheic dermatitis* and *partial* (15–30% activity) biotinidase deficiency, in whom the dermatitis resolved with biotin therapy, have been reported; these children were otherwise asymptomatic. Asymptomatic children and adults with this enzyme deficiency have been identified in screening programs. Most of these individuals have been shown to have partial biotinidase deficiency. With universal newborn screening leading to early identification and treatment of the affected patients, the clinical disease should become extinct.

Laboratory findings and the pattern of organic acids in body fluids resemble those associated with holocarboxylase synthetase deficiency. **Diagnosis** can be established by measurement of the enzyme activity in the serum and confirmed by identifying biallelic pathogenic variants in *BTD*. **Treatment** with free biotin (5-20 mg/day) results in a dramatic clinical and biochemical improvement. Treatment with biotin is also suggested for individuals with partial biotinidase deficiency. The prevalence of this autosomal recessive disorder is estimated to be 1 in 60,000 live births. **Prenatal diagnosis** is possible by identification of known pathogenic variants in *BTD* or, less frequently, by the measurement of the enzyme activity in the amniotic cells, although in practice, a prenatal approach is rarely used.

Multiple Carboxylase Deficiency Caused by Acquired Biotin Deficiency

Acquired deficiency of biotin may occur in infants receiving total parenteral nutrition without added biotin, in patients with prolonged use of antiepileptic drugs (phenobarbital, phenytoin, primidone, carbamazepine), and in children with short bowel syndrome or chronic diarrhea who are receiving formulas low in biotin. Excessive ingestion of raw eggs may also cause biotin deficiency because the protein avidin in egg white binds biotin, decreasing its absorption. Infants with acquired biotin deficiency may develop dermatitis, alopecia, and candidal skin infections. This condition readily responds to treatment with oral biotin.

3-METHYLCROTONYL-COA CARBOXYLASE DEFICIENCY

This enzyme is one of the four carboxylases requiring biotin as a cofactor (see Fig. 105.4). An isolated deficiency of this enzyme must be differentiated from disorders of biotin metabolism (multiple carboxylase deficiency), which causes diminished activity of all four carboxylases (see earlier). 3-Methylcrotonyl-CoA carboxylase (3-MCC) is a heteromeric enzyme consisting of α (biotin containing) and β subunits, encoded by the genes *MCCC1* and *MCCC2,* respectively. 3-MCC deficiency can be detected in the newborn period by identifying elevated 3-hydroxyisovalerylcarnitine (C5-OH) in dried blood spots. Universal newborn screening using tandem mass spectrometry has identified an unexpectedly high number of infants with 3-MCC deficiency, with its prevalence ranging between 1 in 2,400 and 1 in 68,000.

Clinical manifestations are highly variable, ranging from completely asymptomatic adults (including mothers of affected newborn infants), to children presenting with developmental delay without episodes of metabolic decompensation, to patients with seizures, hyperammonemia, and metabolic acidosis. Rarely, infants who are affected by severe 3-MCC deficiency may appear healthy at birth but later develop acute episodes of vomiting, hypotonia, lethargy, and convulsions after a minor infection, in some cases progressing to life-threatening complications (e.g., Reye syndrome, coma). In patients prone to developing these symptoms, the onset is usually between 3 weeks and 3 years of age. Among infants identified through newborn screening, 85–90% of children remain apparently asymptomatic. The reason for differences in outcomes is unknown. None of the symptoms reported so far could be clearly attributed to the degree of enzyme deficiency.

Laboratory findings during acute episodes include mild to moderate metabolic acidosis, ketosis, hypoglycemia, hyperammonemia, and elevated serum transaminase levels. Large amounts of 3-hydroxyisovalerate and 3-methylcrotonylglycine are found in the urine. Urinary excretion of 3-methylcrotonate is usually not detected in this condition because the accumulated 3-methylcrotonyl-CoA is converted to 3-hydroxyisovalerate. The plasma acylcarnitine profile shows elevated 3-hydroxyisovalerylcarnitine (C5-OH). Severe secondary carnitine deficiency is common. 3-MCC deficiency should be differentiated biochemically from multiple carboxylase deficiency (see earlier), in which, in addition to 3-hydroxyisovalerate, lactate and metabolites of propionic acid are also present. **Diagnosis** may be confirmed by molecular analysis of *MCCC1* and *MCCC2* or by measurement of the enzyme activity in cultured fibroblasts. Documentation of normal activities of other carboxylases is necessary to rule out multiple carboxylase deficiency.

Treatment of acute episodes is similar to that of isovaleric acidemia (see earlier). Hydration and measures to correct both hypoglycemia and severe metabolic acidosis by infusing glucose and sodium bicarbonate should be instituted promptly. Secondary carnitine deficiency, seen in up to 50% of patients, can be corrected with L-carnitine supplementation. For symptomatic patients, some centers recommend keeping protein intake at the recommended dietary allowance in conjunction with the oral administration of L-carnitine and the proactive management of catabolic states. Normal physiologic growth and development are expected in most patients.

3-MCC deficiency is an autosomal recessive condition. Biallelic pathogenic variants in either *MCCC1* or *MCCC2* result in the enzyme deficiency with overlapping clinical features.

3-METHYLGLUTACONIC ACIDURIAS

The 3-methylglutaconic acidurias comprise a heterogeneous group of metabolic disorders characterized by excessive excretion of 3-methylglutaconic acid in the urine (Table 105.2). Other metabolites found in 3-methylglutaconic aciduria patients may include 3-methylglutaric acid and 3-hydroxyisovaleric acid. The current classification distinguishes between primary and secondary forms. **Primary** 3-methylglutaconic aciduria is caused by the deficiency of mitochondrial 3-methylglutaconyl-CoA hydratase (see Fig. 105.4), formerly *3-methylglutaconic aciduria type I*. **Secondary** 3-methylglutaconic aciduria can be further classified based on the underlying mechanism (e.g., defective phospholipid remodeling versus dysfunction of mitochondrial membrane) or now the more preferred classification based on the underlying molecular cause. Known causes of secondary 3-methylglutaconic aciduria include the X-linked *TAZ*-related syndrome **(Barth syndrome)**, *OPA3*-related 3-methylglutaconic aciduria **(Costeff syndrome)**, *SERAC1*-related syndrome **(MEGDEL syndrome)**, *TMEM70*-related syndrome, and *DNAJC19*-related syndrome **(DCMA syndrome)**.

Significant and persistent 3-methylglutaconic aciduria with negative molecular evaluation for known genetic causes represents a heterogeneous group called 3-methylglutaconic aciduria **not otherwise specified** awaiting further molecular characterization. Primary and secondary 3-methylglutaconic aciduria should be distinguished from mild and transient urinary elevations of 3-methylglutaconic acid seen in patients affected by other metabolic disorders or liver dysfunction, such as mitochondrial disorders of diverse etiology.

3-Methylglutaconyl-CoA Hydratase Deficiency

Two main clinical presentations have been described for 3-methylglutaconyl-CoA hydratase deficiency, an autosomal recessive disorder (see Fig. 105.4). In the **childhood** form, nonspecific neurodevelopmental findings such as speech delay or regression, choreoathetoid movements, optic nerve atrophy, and mild psychomotor delay may be present. Metabolic acidosis may occur during a catabolic state. In the **adulthood** form, affected individuals may remain asymptomatic until the second or third decade of life, when a clinical picture of *slowly progressing leukoencephalopathy* with optic nerve atrophy, dysarthria, ataxia, spasticity, and dementia occurs. Brain MRI typically shows white matter abnormalities, which may precede the appearance of clinical symptoms by years. Asymptomatic pediatric and adult patients have also been reported. Patients excrete large amounts of 3-methylglutaconic acid and moderate amounts of 3-hydroxyisovaleric and 3-methylglutaric acids in urine. **Treatment** with L-carnitine may help some patients. The effectiveness of a low-leucine diet has not been established. The condition is caused by biallelic pathogenic variants in *AUH* and is inherited in an autosomal recessive manner.

Barth Syndrome (*Tafazzin*-Related Disorder)

This X-linked condition is caused by a deficiency of *tafazzin,* a mitochondrial protein, encoded by the *TAFAZZIN* gene. This enzyme is necessary for remodeling of immature cardiolipin into its mature form. *Cardiolipin,* a mitochondrial phospholipid, is critical for the integrity of the inner mitochondrial membrane. **Clinical manifestations** of Barth syndrome usually begin in the first year of life in a male infant and include cardiomyopathy, hypotonia, growth restriction, hypoglycemia, and mild to severe neutropenia. The onset of clinical manifestations may be as late as adulthood, but most affected individuals become symptomatic by adolescence. If patients survive infancy, relative improvement may occur with advancing age. Cognitive development is usually normal, although delayed motor function and learning disabilities occur.

Laboratory findings include mild to moderate increases in urinary excretion of 3-methylglutaconic, 3-methylglutaric, and 2-ethylhydracrylic acids. Unlike primary 3-methylglutaconic aciduria (type I), urinary excretion of 3-hydroxyisovaleric acid is not elevated. The activity of the enzyme 3-methylglutaconyl-CoA hydratase is normal. *Neutropenia is a common finding.* Lactic acidosis, hypoglycemia, low serum cholesterol, low prealbumin, and abnormal mitochondrial ultrastructure are also common. Total cardiolipin and subclasses of cardiolipin are very low in skin fibroblast cultures from these patients. The monolysocardiolipin/cardiolipin ratio in cultured fibroblast is useful for establishing the diagnosis in patients with negative or equivocal molecular results. Because of its nonspecific presentation, the condition is likely underdiagnosed and underreported.

The condition is inherited in an *X-linked* recessive manner. The gene *(TAFAZZIN)* has been mapped to chromosome Xq28. The modest 3-methylglutaconic aciduria seen in Barth syndrome is thought to be related to the defect in the mitochondrial membrane, causing the leakage of this organic acid. Gene-specific treatment is not available. Patients with an unsatisfactory response to medical management of cardiomyopathy may benefit from cardiac transplantation. Daily aspirin to reduce the risk of strokes has been described. Regular use of granulocyte colony stimulating factor (G-CSF) can help limit recurrent infections in patients with neutropenia.

OPA3-Related 3-Methylglutaconic Aciduria (Costeff Syndrome)

Clinical manifestations in patients with Costeff syndrome include early-onset optic nerve atrophy and later development of choreoathetoid movements, spasticity, ataxia, dysarthria, and cognitive impairment. Patients excrete moderate amounts of 3-methylglutaconic and 3-methylglutaric acids. Activity of the enzyme 3-methylglutaconyl-CoA hydratase is normal. The condition is inherited in an autosomal recessive manner. Pathogenic variants in *OPA3* are thought to cause electron transport chain dysfunction. Treatment is supportive.

Disorders Formerly Described as 3-Methylglutaconic Aciduria Type IV

3-Methylglutaconic aciduria type IV represents a group of disorders with a diverse genetic etiology. Two disorders in this group have been linked to specific molecular etiology, whereas other conditions are still awaiting the discovery of underlying molecular defects.

MEGDEL syndrome (3-*me*thylglutaconic aciduria with *d*eafness, *e*ncephalopathy, and *L*eigh-like) is an autosomal recessive disorder caused by pathogenic variants in *SERAC1*. Affected patients experience optic nerve atrophy, progressive deafness, dystonia, feeding difficulty with dysphagia, spasticity, and basal ganglia injury similar to patients

Table 105.2 Primary and Select Secondary 3-Methylglutaconic Acidurias

GROUP	DISORDER	GENE (CHROMOSOME)	PREVIOUS CLASSIFICATION	DISEASE MECHANISM	CLINICAL DESCRIPTION
Primary 3-methylglutaconic aciduria	3-Methylglutaconyl-CoA hydratase deficiency	*AUH* (9q22.31)	Type I	Enzyme deficiency in the leucine degradation pathway	Depending on age, variable presentation is seen ranging from younger asymptomatic patients to older patients with progressive leukoencephalopathy
Secondary 3-methylglutaconic acidurias	Barth syndrome	*TAZ* (Xq28)	Type II	Defective phospholipid remodeling	X-linked inheritance, cardiomyopathy, endocardial fibroelastosis, proximal myopathy, failure to thrive, neutropenia, dysmorphic findings
	Costeff syndrome	*OPA3* (19q13.32)	Type III	Mitochondrial membrane dysfunction	Progressive optic nerve atrophy, chorea, spastic paraparesis, cognitive impairment
	MEGDEL syndrome	*SERAC1* (6q25.3)	Type IV	Defective phospholipid remodeling	Progressive deafness, dystonia, spasticity, basal ganglia changes
	TMEM70-related disorder	*TMEM70* (8q21.11)	Type IV	Mitochondrial membrane dysfunction	Developmental delay, failure to thrive, metabolic decompensations, microcephaly, cardiomyopathy, dysmorphic findings
	3-Methylglutaconic aciduria, not otherwise specified	Unknown	Type IV	Unknown	Variable presentation
	DCMA syndrome	*DNAJC19* (3q26.33)	Type V	Mitochondrial membrane dysfunction	Cardiomyopathy, ataxia, optic nerve atrophy, failure to thrive

DCMA, Dilated cardiomyopathy ataxia syndrome; MEGDEL, 3-methylglutaconic aciduria deafness, encephalopathy, Leigh-like syndrome.

with Leigh syndrome. Laboratory evaluation reveals elevated urinary 3-methylglutaconic, high plasma lactate and alanine. Treatment is symptomatic.

***TMEM70*-related disorder** is also inherited in an autosomal recessive manner. Biallelic pathogenic variants in *TMEM70* result in a mitochondrial complex V deficiency, although the exact molecular mechanism of disease is unknown. Clinical manifestations include developmental delay, developmental regression, Reye syndrome–like episodes, intellectual disability, failure to thrive, microcephaly, cardiomyopathy, and dysmorphic findings. Patients are prone to metabolic decompensation, characterized by hyperammonemia (up to 900 µmol/L) and lactic acidosis, which are more common in the first year of life. **Acute hyperammonemic** episodes are treated with intravenous glucose, lipid emulsion, ammonia-scavenging drugs, and occasionally hemodialysis. Long-term therapy that has been described includes L-carnitine, coenzyme Q_{10}, and bicarbonate substitution (e.g., citric acid/sodium citrate). Patients require interval echocardiographic and electrocardiographic (ECG) monitoring to enable early diagnosis and management of cardiomyopathy.

DCMA Syndrome (*DNAJC19*-Related Syndrome, 3-Methylglutaconic Aciduria Type V)

DCMA syndrome (*d*ilated *c*ardio*m*yopathy with *a*taxia) is an autosomal recessive disorder identified in patients of Canadian Dariusleut Hutterite ancestry in the Great Plains of North America. As the disorder's abbreviated name suggests, affected individuals present with dilated cardiomyopathy, long QTc interval, and CNS involvement. Neurologic symptoms include intellectual disability, cerebellar involvement, and optic atrophy. Growth is affected in all patients. Intrauterine growth restriction is seen in up to 50% of patients. Cryptorchidism and hypospadias are frequent findings in affected boys. Urine organic acid assay reveals increased 3-methylglutaconic acid and 3-methylglutaric acid. Biallelic pathogenic variants in *DNAJC19* are the underlying cause of DCMA syndrome. Treatment is symptomatic. Interval echocardiography and ECG can prospectively identify patients requiring treatment for cardiomyopathy and long QTc interval.

β-KETOTHIOLASE (3-OXOTHIOLASE) DEFICIENCY (MITOCHONDRIAL ACETOACETYL-COA THIOLASE [T_2] DEFICIENCY)

The bidirectional reaction catalyzed by mitochondrial β-ketothiolase is involved in the final steps of both isoleucine catabolism and ketolysis. In the isoleucine catabolic pathway, the enzyme cleaves 2-methylacetoacetyl-CoA into propionyl-CoA and acetyl-CoA (see Fig. 105.4). In the fatty acid oxidation pathway, the enzyme generates two moles of acetyl-CoA from one mole of acetoacetyl-CoA (Fig. 105.7). The same enzyme can synthesize 2-methylacetoacetate-CoA and acetoacetyl-CoA in the reverse direction. The hallmark of this disorder is **ketoacidosis**, often triggered by infections, prolonged fasting, and large protein load. The mechanism of ketosis in this condition is incompletely understood, because in this enzyme deficiency one would expect impaired ketone formation (see Fig. 105.7). It is postulated that excess acetoacetyl-CoA produced from other sources can be used as a substrate for 3-hydroxy-3-methylglutaryl-CoA synthesis in the liver.

Clinical manifestations are quite variable, ranging from mild cases showing normal development to severe episodes of acidosis starting in the first year of life causing severe cognitive impairment. Unless identified on the newborn screening, affected children present with intermittent episodes of unexplained ketoacidosis. These episodes usually occur after an intercurrent infection and respond promptly to

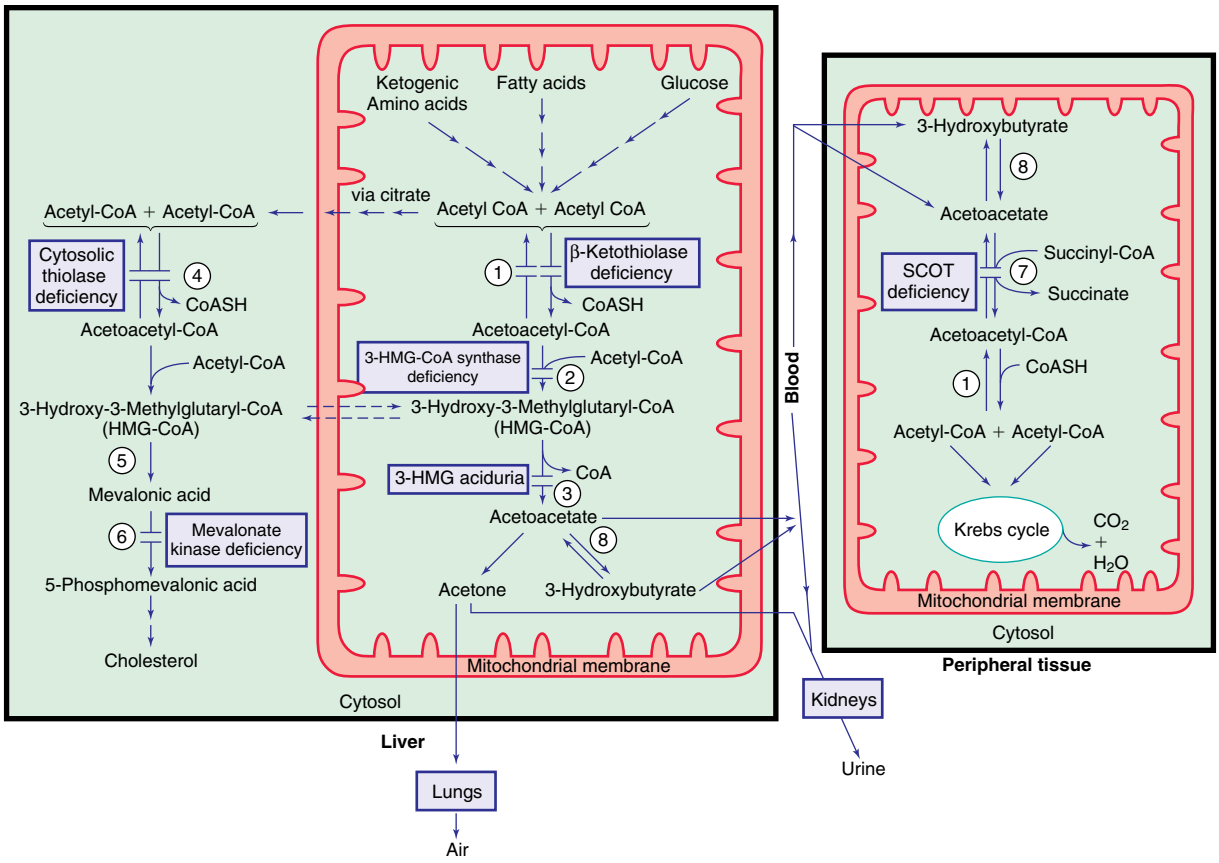

Fig. 105.7 Formation (liver) and metabolism (peripheral tissues) of ketone bodies and cholesterol synthesis. **Enzymes:** *(1)* Mitochondrial acetoacetyl CoA thiolase, *(2)* HMG-CoA synthase, *(3)* HMG-CoA lyase, *(4)* cytosolic acetoacetyl-CoA thiolase, *(5)* HMG-CoA reductase, *(6)* mevalonic kinase, *(7)* succinyl CoA:3-ketoacid CoA transferase (SCOT), *(8)* 3-hydroxybutyrate dehydrogenase.

intravenous fluids and bicarbonate therapy. Mild to moderate hyperammonemia may also be present during attacks. Both hypoglycemia and hyperglycemia have been reported in isolated cases. The child may be completely asymptomatic between episodes and may tolerate a normal protein diet. Cognitive development is normal in most children. The episodes may be misdiagnosed as salicylate poisoning because of the similarity of the clinical findings and the interference of elevated blood levels of acetoacetate with the colorimetric assay for salicylate.

Laboratory findings during the acute attack include ketoacidosis and hyperammonemia. Findings of ketones in the urine and hyperglycemia may be interpreted as diabetic ketoacidosis, and a high index of suspicion is needed to suspect this metabolic disorder. Urine organic acid assay can provide clues leading to a correct diagnosis. Urine contains large amounts of 2-methylacetoacetate and its decarboxylated products butanone, 2-methyl-3-hydroxybutyrate, and tiglylglycine. Lower concentrations of urinary metabolites can be seen when patients are stable. Mild hyperglycinemia may also be present. The plasma acylcarnitine profile shows elevations of C5:1 and C5-OH carnitines, although these metabolites can normalize between catabolic episodes. Absent or minimal elevations of C5:1 and C5-OH carnitines can result in false-negative results on the newborn screening of affected infants who were clinically well at the time of blood collection. The clinical and biochemical findings should be differentiated from those seen with propionic and methylmalonic acidemias (see later).

Treatment of acute episodes includes hydration. Recalcitrant metabolic acidosis can be severe enough to require infusion of bicarbonate. A 10% glucose solution with the appropriate electrolytes is used to suppress protein catabolism, lipolysis, and ketogenesis. Restriction of protein intake to age-appropriate physiologic requirements is recommended for long-term therapy. Oral L-carnitine (50-100 mg/kg/day) is also recommended to prevent possible secondary carnitine deficiency. The long-term prognosis for achieving a normal quality of life seems very favorable. Successful pregnancy with a normal outcome has been reported.

β-Ketothiolase deficiency is inherited in an autosomal recessive manner and may be more prevalent than appreciated. **Diagnosis** may be confirmed by molecular analysis of the *ACAT1* gene or using an enzyme assay of leukocytes or cultured fibroblasts.

CYTOSOLIC ACETOACETYL-COA THIOLASE DEFICIENCY

This enzyme catalyzes the cytosolic production of acetoacetyl-CoA from two moles of acetyl-CoA (see Fig. 105.7). Cytosolic acetoacetyl-CoA is the precursor of hepatic cholesterol synthesis. Cytosolic acetoacetyl-CoA thiolase (encoded by *ACAT2*) should be differentiated from the mitochondrial thiolase (see earlier and Fig. 105.4). Clinical manifestations in patients with this very rare enzyme deficiency have been incompletely characterized. Patients may present with severe progressive developmental delay, hypotonia, and choreoathetoid movements in the first few months of life. Laboratory findings are nonspecific; elevated levels of lactate, pyruvate, acetoacetate, and 3-hydroxybutyrate may be found in blood and urine. One patient had normal levels of acetoacetate and 3-hydroxybutyrate. Diagnosis can be aided by demonstrating a deficiency in cytosolic thiolase activity in liver biopsy or in cultured fibroblasts or by DNA analysis of *ACAT2*. No effective treatment has been described, although a low-fat diet helped to diminish ketosis in one patient.

MITOCHONDRIAL 3-HYDROXY-3-METHYLGLUTARYL-COA SYNTHASE DEFICIENCY

3-Hydroxy-3-methylglutaryl (HMG)-CoA synthase (mitochondrial or type 2) catalyzes synthesis of 3-HMG-CoA from acetoacetyl-CoA and acetyl-CoA in the mitochondria. This is a critical step in ketone body synthesis in the liver (see Fig. 105.7). Only a few patients with

deficiency of this enzyme have been reported. The principal clinical syndrome is *hypoketotic hypoglycemia* triggered by physiologic stress, such as infections or fasting. Age at presentation has ranged from infancy to 6 years. Children tend to be asymptomatic before these episodes and with appropriate management can remain stable after the recovery (except for mild hepatomegaly with fatty infiltration). Future episodes can be prevented by avoiding prolonged fasting during ensuing intercurrent illnesses. Hepatomegaly is the most consistent physical finding in these patients. **Laboratory findings** include hypoglycemia, acidosis with mild or no ketosis, elevated levels in liver function tests, and massive dicarboxylic aciduria. The clinical and laboratory findings may be confused with fatty acid metabolism defects (see Chapter 104). In contrast to the latter, in patients with HMG-CoA synthase deficiency, the blood concentrations of acylcarnitine conjugates are negative for acylcarnitine findings characteristic of fatty acid oxidation disorders. Treatment of the secondary carnitine deficiency with L-carnitine supplementation can result in elevated plasma acetylcarnitine (C2-carnitine), likely reflecting intracellular accumulation of acetyl-CoA.

Treatment consists of provision of adequate calories and avoidance of prolonged periods of fasting. No dietary protein restriction was needed.

The condition is inherited in an autosomal recessive manner and is caused by biallelic pathogenic variants in *HMGCS2*. The condition should be considered in any child with fasting hypoketotic hypoglycemia and may be more common than appreciated.

3-HYDROXY-3-METHYLGLUTARYL-COA LYASE DEFICIENCY (3-HYDROXY-3-METHYLGLUTARIC ACIDURIA)

3-HMG-CoA lyase (encoded by *HMGCL*) catalyzes the cleavage of 3-HMG-CoA to acetoacetate, a rate-limiting enzyme for ketogenesis (see Fig. 105.4 and Fig. 105.7). The deficiency of this enzyme is a rare disorder seen with increased frequency in Saudi Arabia, the Iberian Peninsula, and in Brazil in patients of Portuguese ancestry. Clinically, approximately 30% develop symptoms in the first few days of life, and >60% of patients become symptomatic between 3 and 11 months of age. Infrequently, patients may remain asymptomatic until adolescence. With the addition of 3-HMG-CoA lyase deficiency to the newborn screening using C5-OH-carnitine, many infants are identified presymptomatically in the newborn period. Similar to 3-HMG-CoA synthase deficiency, patients affected by 3-HMG-CoA lyase deficiency may present with acute hypoketotic hypoglycemia. Episodes of vomiting, severe hypoglycemia, hypotonia, acidosis with mild or no ketosis, and dehydration may rapidly lead to lethargy, ataxia, and coma. These episodes often occur during a catabolic state such as prolonged fasting or an intercurrent infection. Hepatomegaly is common. These manifestations may be mistaken for Reye syndrome or fatty acid oxidation defects such as medium-chain acyl-CoA dehydrogenase deficiency. Long-term complications can include dilated cardiomyopathy, hepatic steatosis, and pancreatitis. Development can be normal, but intellectual disability and seizures with abnormalities in the white matter seen on brain MRI have been observed in patients after prolonged episodes of hypoglycemia.

Laboratory findings include hypoglycemia, moderate to severe hyperammonemia, and acidosis. There is mild or no ketosis (see Fig. 105.7). Urinary excretion of 3-hydroxy-3-methylglutaric acid and other proximal intermediate metabolites of leucine catabolism (3-methylglutaric acid, 3-methylglutaconic acid, and 3-hydroxyisovaleric acid) is markedly increased, causing the urine to smell like "cat's urine." Glutaric and dicarboxylic acids may also be increased in urine during acute attacks. Secondary carnitine deficiency is common. The condition is inherited in an autosomal recessive manner. 3-HMG-CoA lyase is encoded by the gene *HMGCL*. **Diagnosis** may be confirmed by molecular analysis of *HMGCL* or by enzyme assay in cultured fibroblasts, leukocytes, or liver specimens. Prenatal diagnosis is possible by molecular DNA analysis if the familial pathogenic variants are known or by enzymatic assay of the cultured amniocytes or a chorionic villi biopsy.

Treatment of acute episodes includes hydration, infusion of glucose to control hypoglycemia, provision of adequate calories, and administration of bicarbonate to correct acidosis. Hyperammonemia should be treated promptly (see Chapter 105.12). Dialysis may be required in patients with severe recalcitrant hyperammonemia. Restriction of protein and fat intake can be considered for long-term management. Oral administration of L-carnitine (50-100 mg/kg/day) prevents secondary carnitine deficiency. Prolonged fasting should be avoided.

SUCCINYL-COA:3-OXOACID-COA TRANSFERASE DEFICIENCY

Succinyl-CoA:3-oxoacid-CoA transferase **(SCOT)** deficiency and β-ketothiolase deficiency collectively are referred to as **ketone utilization disorders**. SCOT participates in the conversion of ketone bodies (acetoacetate and 3-hydroxybutyrate) generated in liver mitochondria into *acetoacetyl-CoA in the nonhepatic tissues* (see Fig. 105.7). A deficiency of this enzyme results in the accumulation of ketone bodies, ketoacidosis, increased use of glucose, and hypoglycemia. During fasting, patients tend to have a proportional elevation of plasma free fatty acids. The condition may be more common than recognized because many cases tend to be mild and remain undiagnosed. SCOT deficiency can be distinguished from β-ketothiolase deficiency by the absence of 2-methylacetoacetate, 2-methyl-3-hydroxybutyrate, and tiglylglycine, characteristic of the latter disorder. Plasma acylcarnitine profile tends to show no specific abnormalities.

A common clinical presentation is an acute episode of severe ketoacidosis in an infant who had been growing and developing normally. About half the patients become symptomatic in the first weeks of life, and practically all become symptomatic before 2 years of age. The acute episode is often precipitated by a catabolic state triggered by an infection or prolonged fasting. Without treatment, the ketoacidotic episode can result in death. A chronic subclinical ketosis may persist between the attacks. Development is usually normal, although severe and recurrent episodes of ketoacidosis and hypoglycemia can predispose patients to neurocognitive impairment.

Laboratory findings during the acute episode are nonspecific and include metabolic acidosis and ketonuria with high levels of acetoacetate and 3-hydroxybutyrate in blood and urine. No other organic acids are found in the blood or in the urine. Blood glucose levels are usually normal, but hypoglycemia has been reported in some affected newborn infants with severe ketoacidosis. Plasma amino acids and the plasma acylcarnitine profile are usually normal. Severe SCOT deficiency can be accompanied by ketosis even when patients are clinically stable. This condition should be considered in any infant with unexplained bouts of ketoacidosis. SCOT deficiency is an autosomal recessive disorder. **Diagnosis** can be established by molecular analysis of *OXCT1* or by demonstrating a deficiency of enzyme activity in cultured fibroblasts.

Treatment of acute episodes consists of rehydration with solutions containing dextrose, correction of acidosis, and the provision of a diet adequate in calories. Long-term treatment should include a high-carbohydrate diet and avoidance of prolonged fasting and administration of dextrose before anticipated or during established catabolic states.

MEVALONATE KINASE DEFICIENCY

Mevalonic acid, an intermediate metabolite of cholesterol synthesis, is converted to 5-phosphomevalonic acid by the action of the enzyme mevalonate kinase (MVK) (see Fig. 105.7). MVK deficiency presents with a range of symptoms. Mevalonic aciduria occupies the more severe end of the spectrum, whereas hyperimmunoglobulinemia D syndrome represents the milder form of the underlying enzyme defect. Both disorders are accompanied by recurrent fever, gastrointestinal symptoms, mucocutaneous manifestations, and lymphadenopathy. Patients with mevalonic aciduria also show growth retardation and nervous system involvement.

Mevalonic Aciduria

Clinical manifestations include failure to thrive, growth restriction, intellectual disability, hypotonia, ataxia, myopathy, hepatosplenomegaly,

cataracts, and facial dysmorphisms (dolichocephaly, frontal bossing, low-set ears, downward slanting of eyes, long eyelashes). Most patients experience recurrent crises characterized by fever, vomiting, diarrhea, hepatosplenomegaly, arthralgia, lymphadenopathy, edema, and morbilliform rash. These episodes typically last 2-7 days and recur up to 25 times a year. Death may occur during these crises.

Laboratory findings include marked elevation of mevalonic acid in urine; the concentration of urinary mevalonic acid ranges between 500 and 56,000 mmol/mol of creatinine (normal: <0.3 mmol/mol of creatinine). Plasma levels of mevalonic acid are also greatly increased (as high as 540 μmol/L; normal: <0.04 μmol/L). Mevalonic acid levels tend to correlate with the severity of the condition and increase during crises. Serum cholesterol concentration is normal or mildly decreased. Serum concentration of creatine kinase can be greatly increased. Inflammatory markers are elevated during the crises. Brain MRI may reveal progressive atrophy of the cerebellum.

Diagnosis may be confirmed by molecular analysis of *MVK* or by assaying the MVK activity in lymphocytes or cultured fibroblasts. The enzyme activity in this form of the condition is below the detection level. **Treatment** with high doses of prednisone helps in the acute crises, but because of side effects, it is not routinely used long term. TNF-α inhibitors and interleukin-1 receptor antagonists have shown to be effective in bringing significant clinical improvement, especially in patients with chronic inflammation and frequent attacks. The condition is inherited in an autosomal recessive manner. **Prenatal diagnosis** is possible by identifying known familial pathogenic variants in *MVK,* by measurement of mevalonic acid in the amniotic fluid, or by assaying the enzyme activity in cultured amniocytes or chorionic villi samples.

Hyperimmunoglobulinemia D Syndrome (Hyperimmunoglobulinemia D and Periodic Fever Syndrome)

Some pathogenic variants of the *MVK* gene cause milder enzyme deficiency and produce the clinical picture of **periodic fever with hyperimmunoglobulinemia D**. These patients have periodic bouts of fever associated with abdominal pain, vomiting, diarrhea, arthralgia, arthritis, hepatosplenomegaly, lymphadenopathy, and morbilliform rash (even petechiae and purpura), which usually start before 1 year of age. The attacks can be triggered by vaccination, minor trauma, or stress and can occur every 1-2 months, lasting 2-7 days. Patients are free of symptoms between acute attacks. The diagnostic laboratory finding is elevation of serum immunoglobulin D (IgD). IgA is also elevated in 80% of patients. During acute attacks, leukocytosis, increased C-reactive protein, and mild mevalonic aciduria may be present. High concentrations of serum IgD help differentiate this condition from familial Mediterranean fever. See Chapter 204 for treatment recommendations.

PROPIONIC ACIDEMIA (PROPIONYL-COA CARBOXYLASE DEFICIENCY)

Propionic acid is an intermediate metabolite of isoleucine, valine, threonine, methionine, odd-chain fatty acids, and side chains of cholesterol. Normally, propionic acid in the form of propionyl-CoA undergoes carboxylation to D-methylmalonyl-CoA, catalyzed by the mitochondrial enzyme propionyl-CoA carboxylase. This enzyme requires biotin as a cofactor; thus the disorders of biotin metabolism, among other findings, can also result in elevation of propionic acid metabolites (see Fig. 105.4). Propionyl-CoA carboxylase is a multimeric enzyme composed of two nonidentical subunits, α and β, encoded by two genes, *PCCA* and *PCCB,* respectively. Biallelic pathogenic variants in propionyl-CoA carboxylase result in an autosomal recessive disorder called *propionic acidemia.*

Clinical findings of propionic acidemia are not specific to this disorder only. In the severe form, patients develop symptoms in the first few days of life. Poor feeding, vomiting, hypotonia, lethargy, dehydration, a sepsis-like picture, and clinical signs of severe ketoacidosis progress rapidly to coma and death. Seizures occur in approximately 30% of affected infants. If an infant survives the first attack, similar episodes of metabolic decompensation may occur during an intercurrent infection, trauma, surgery, prolonged fasting, severe constipation, or after ingestion of a high-protein diet. Moderate to severe intellectual disability and neurologic manifestations reflective of extrapyramidal (dystonia, choreoathetosis, tremor) and pyramidal (paraplegia) dysfunction are common sequelae in survivors. Neuroimaging shows that these abnormalities, which often occur after an episode of metabolic decompensation, are the result of damage to the basal ganglia, especially to the globus pallidus. This phenomenon has been referred to as **metabolic stroke**. This is the main cause of neurologic sequelae seen in the surviving affected children. Additional long-term complications include failure to thrive, optic nerve atrophy, pancreatitis, cardiomyopathy, and osteopenia.

In the *milder* form, episodes of metabolic decompensation are less frequent, but these children are still at risk of developing intellectual disability, seizures, long QTc interval, and severe cardiomyopathy. Universal newborn screening can identify propionic acidemia by detecting elevated propionylcarnitine (C3-carnitine) with an abnormal C3/C2 ratio in dried blood spots. However, in patients with the mild form of propionic acidemia, propionylcarnitine may remain below the cutoff value set by the screening laboratory, resulting in a false-negative result. Therefore physicians should maintain a high index of suspicion for this disorder and follow up with a biochemical evaluation of infants and children presenting with unexplained ketosis or metabolic acidosis.

Laboratory findings during the acute attack include various degrees of metabolic acidosis, often with a large anion gap, ketosis, ketonuria, hypoglycemia, anemia, neutropenia, and thrombocytopenia. Moderate to severe hyperammonemia is common; plasma ammonia concentrations usually correlate with the severity of the disease. In contrast to other causes of hyperammonemia, plasma concentration of glutamine tends to be within normal limits or decreased. The presence of severe metabolic acidosis and normal to reduced plasma glutamine help differentiate propionic academia from hyperammonemia caused by urea cycle defects. Measurement of plasma ammonia is especially helpful in planning therapeutic strategies during episodes of exacerbation in a patient whose diagnosis has been established. Mechanisms of hyperammonemia in propionic acidemia are not well understood but are likely related to the perturbed biochemical and pH environment of the mitochondrial matrix, where the proximal part of the urea cycle resides.

Glycine concentration can be elevated in all body fluids (blood, urine, CSF) and possibly is the result of the inhibited glycine cleavage system in the hepatic mitochondria (Fig. 105.8). Glycine elevation has also been observed in patients with methylmalonic acidemia. These disorders were collectively referred to as *ketotic hyperglycinemia* in the past before the specific enzyme deficiencies were elucidated. Mild to moderate increase in blood lactate and lysine may also be present in these patients. Concentrations of propionylcarnitine, 3-hydroxypropionic acid, and methylcitric acid (presumably formed through condensation of propionyl-CoA with oxaloacetic acid) are greatly elevated in the plasma and urine of infants with propionic acidemia. Propionylglycine and other intermediate metabolites of BCAA catabolism, such as tiglylglycine, can also be found in urine. Moderate elevations in blood levels of glycine and previously mentioned organic acids can persist between the acute attacks. Brain imaging may reveal cerebral atrophy, delayed myelination, and abnormalities in the globus pallidus and other parts of the basal ganglia.

The **diagnosis** of propionic acidemia should be differentiated from multiple carboxylase deficiencies (see earlier and Fig. 105.6). In addition to propionic acid metabolites, infants with the latter condition excrete large amounts of lactic acid, 3-methylcrotonylglycine, and 3-hydroxyisovaleric acid. The presence of hyperammonemia may suggest a genetic defect in the urea cycle enzymes. Infants with defects in the urea cycle are usually *not* acidotic (see Fig. 105.1) and have elevated levels of plasma glutamine. The definitive diagnosis of propionic acidemia can be established through molecular analysis of *PCCA* and *PCCB* or by measuring the enzyme activity in leukocytes or cultured fibroblasts.

Treatment of acute episodes of metabolic decompensation includes hydration with solutions containing glucose, correction of acidosis, and amelioration of the catabolic state by provision of adequate calories through enteral or parenteral hyperalimentation. A brief restriction of

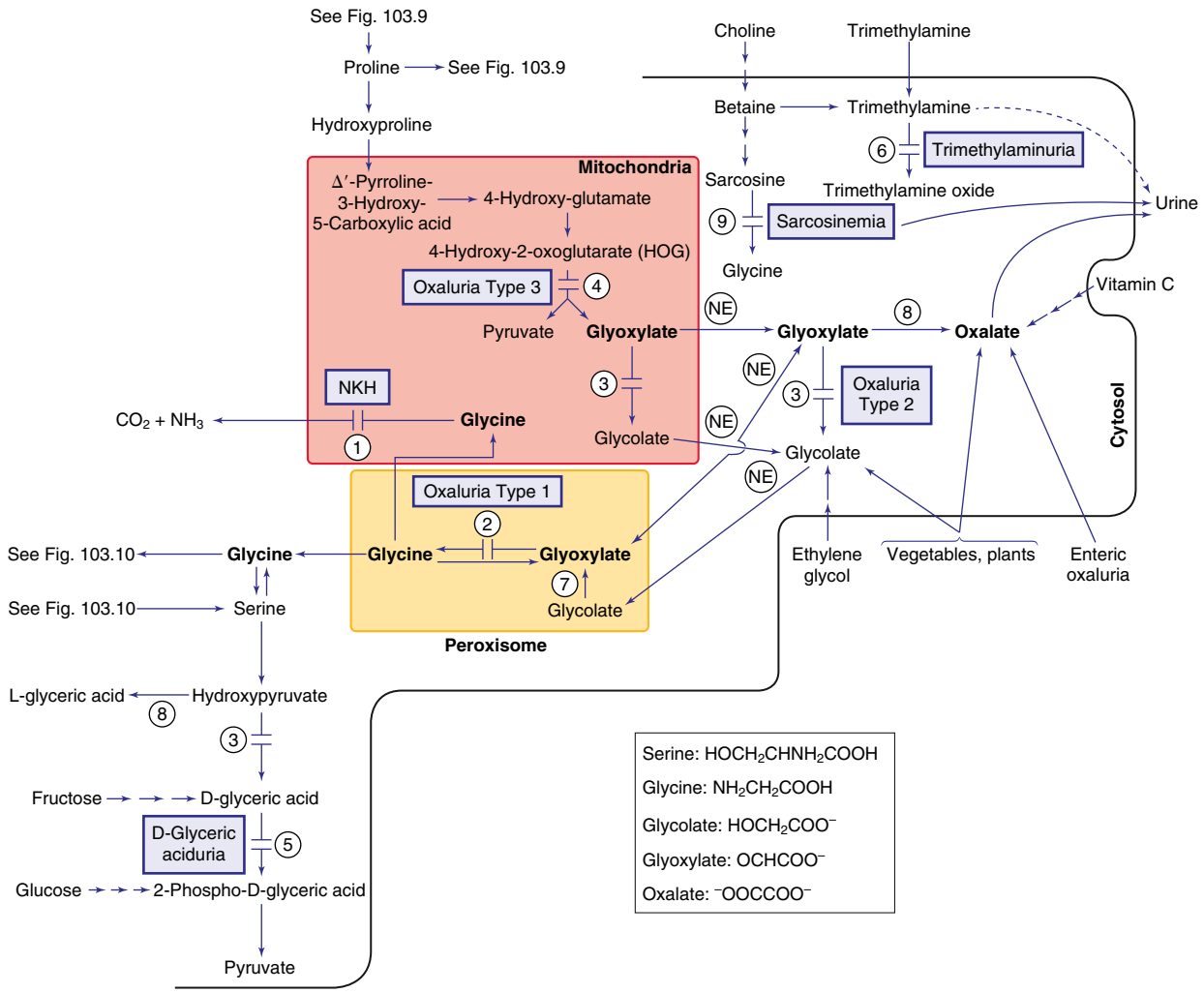

Fig. 105.8 Pathways in the metabolism of glycine and glyoxylic acid. **Enzymes:** *(1)* Glycine cleavage system, *(2)* alanine:glyoxylate aminotransferase, *(3)* glyoxylic reductase/hydroxypyruvate reductase (GR/HRP), *(4)* hydroxyoxoglutarate aldolase one (HOGA1), *(5)* glycerate kinase, *(6)* trimethylamine oxidase, *(7)* glycolate oxidase (D-amino acid oxidase), *(8)* lactate dehydrogenase, *(9)* sarcosine dehydrogenase. NE, Nonenzymatic; NKH, nonketotic hyperglycinemia.

protein intake, no more than 24 hours, is often necessary. Depending on the clinical status, gradual reintroduction of protein is recommended. If enteral feedings cannot be tolerated after 48 hours of protein restriction, parenteral nutrition should be instituted to achieve the age-specific recommended protein intake. Patients unable to tolerate the recommended dietary allowance of protein can receive specialized medical foods free of isoleucine, valine, threonine, and methionine. The composition and amount of protein typically vary among patients. The metabolic diet composition can be adjusted by monitoring growth and plasma amino acids drawn 3-4 hours after the typical feeding. Some patients may benefit from the suppression of propionogenic gut microflora. This can be achieved by oral antibiotics such as oral neomycin or metronidazole. Prolonged use of metronidazole should be avoided because it has been associated with reversible peripheral neuropathy and increased QTc interval. The risk of QTc prolongation can be problematic in propionic acidemia patients, who are at risk for cardiomyopathy and long QT interval. Baseline and interval ECGs are recommended before and after initiation of metronidazole therapy. Patients may benefit from management of constipation.

Patients with propionic acidemia often develop *secondary carnitine deficiency,* presumably as a result of the urinary loss of propionylcarnitine. Administration of L-carnitine (50-100 mg/kg/day orally or intravenously) helps restore free carnitine in blood. In patients with concomitant hyperammonemia, measures to reduce blood ammonia should be employed (see Chapter 105.12). Very ill patients with severe acidosis and hyperammonemia require hemodialysis to remove ammonia and other toxic compounds rapidly and efficiently. Carglumic acid and nitrogen scavengers (sodium benzoate, sodium phenylacetate, sodium phenylbutyrate) can aid in the treatment of acute hyperammonemia. Although no infant with propionic acidemia has been found to be responsive to biotin, this compound should be administered (10 mg/day orally) to all infants during the first attack and until the diagnosis is established and multiple carboxylase deficiency ruled out.

Long-term treatment consists of a low-protein diet meeting age-specific recommended dietary allowance and administration of L-carnitine (50-100 mg/kg/day orally). Some centers manage mild cases of propionic acidemia without medical foods, opting for only restricting the protein intake to the recommended dietary allowance. Patients unable to tolerate the recommended dietary intake of protein may require medical foods free of propionate precursors (isoleucine, valine, methionine, and threonine). Excessive use of medical foods while restricting natural-source protein may cause a deficiency of the essential amino acids, especially isoleucine and valine, which may cause a condition resembling **acrodermatitis enteropathica** (see Chapter 712). Over-restriction of methionine, especially in the first years of life, may contribute to the reduced brain growth and microcephaly. To avoid this problem, natural proteins should comprise most of the dietary protein. Some patients may require bicarbonate substitution (e.g., citric acid/sodium citrate) to correct chronic acidosis. The concentration of plasma ammonia usually normalizes between attacks, although

some patients may experience mild chronic hyperammonemia. Acute attacks triggered by infections, fasting, trauma, stress, constipation, or dietary indiscretions should be treated promptly and aggressively. Close monitoring of plasma ammonia, plasma amino acids obtained 3-4 hours after the last typical meal (especially isoleucine, leucine, valine, threonine, and methionine), and growth parameters is necessary to ensure the diet is appropriate. Orthotopic liver transplantation is used in clinically unstable patients experiencing recurrent hyperammonemia, frequent metabolic decompensations, and poor growth. Liver transplantation does not cure propionic acidemia, and lifelong dietary management and proactive management during periods of significant metabolic stress are recommended.

The long-term **prognosis** is poor. Death may occur during an acute attack. Normal psychomotor development is possible in a mild form identified through newborn screening. Children identified clinically may manifest some degree of permanent neurodevelopmental deficit, such as tremor, dystonia, chorea, and spasticity despite adequate therapy. These neurologic findings may be the sequelae of a metabolic stroke occurring during an acute decompensation. A long QTc interval and cardiomyopathy with potential progression to heart failure, fatal arrhythmias, and death may develop in older affected children despite adequate metabolic control. Acute pancreatitis is a common and severe complication in propionic acidemia. Osteoporosis can predispose to fractures, which can occur with minimal mechanical stress.

Prenatal diagnosis can be achieved by identification of known familial pathogenic variants in *PCCA* or *PCCB* or by measuring the enzyme activity in cultured amniotic cells or in samples of uncultured chorionic villi.

Propionic acidemia is inherited in an autosomal recessive manner. It has a worldwide prevalence of 1 in 105,000 to 1 in 250,000 live births. It is more prevalent in Greenlandic Inuits (1 in 1,000) and in some Saudi Arabian tribes (1 in 2,000 to 1 in 5,000 live births). Biallelic pathogenic variants in either gene result in similar clinical and biochemical manifestations. Although pregnancies with normal outcomes have been reported, the perinatal period poses special risks to females with propionic acidemia because of hyperemesis gravidarum, worsening cardiomyopathy, changing protein requirements, and risk of metabolic decompensation.

ISOLATED METHYLMALONIC ACIDEMIAS

Methylmalonic acidemias are a group of metabolic disorders of diverse etiology characterized by impaired conversion of methylmalonyl-CoA into succinyl-CoA. Propionyl-CoA derived from catabolism of isoleucine, valine, threonine, methionine, side chain of cholesterol, and odd-chain fatty acids is catalyzed by propionyl-CoA carboxylase to form D-methylmalonyl-CoA. Methylmalonyl-CoA epimerase then converts D-methylmalonyl-CoA to its enantiomer L-methylmalonyl-CoA. **Methylmalonyl-CoA epimerase deficiency** is a rare disorder associated with persistent elevations of propionate-related metabolites and methylmalonic acid. It may present with metabolic acidosis, ketosis, but patients appear more clinically stable than those with severe forms of methylmalonic acidemia.

In the next biochemical step, L-methylmalonyl-CoA is converted to succinyl-CoA by methylmalonyl-CoA mutase (see Fig. 105.4). The latter enzyme requires 5-deoxyadenosylcobalamin, a metabolite of vitamin B_{12}, as a coenzyme. Deficiency of either the mutase or its coenzyme results in the accumulation of methylmalonic acid and its precursors in body fluids. Two biochemical forms of methylmalonyl-CoA mutase deficiencies have been identified. These are designated *mut*^{0}, referring to no detectable enzyme activity, and *mut*$^-$, indicating residual, although insufficient, mutase activity. Patients with methylmalonic acidemia due to deficiency of the mutase apoenzyme (*mut*^{0}) are not responsive to hydroxocobalamin therapy.

In the remaining methylmalonic acidemia patients, the defect resides in the formation of adenosylcobalamin from dietary vitamin B_{12}. The absorption of dietary vitamin B_{12} in the terminal ileum requires *intrinsic factor,* a glycoprotein secreted by the gastric parietal cells. It is transported in the blood by haptocorrin and transcobalamin II. The transcobalamin II–cobalamin complex (TCII-Cbl) is recognized by a specific receptor on the cell membrane (a transcobalamin receptor encoded by *CD320*) and enters the cell by endocytosis. In the lysosome, TCII-Cbl is hydrolyzed, and, with the participation of LMBRD1 (*cbl*F) and ABCD4 (*cbl*J), free cobalamin is released into the cytosol (see Fig. 105.4). Biallelic pathogenic variants in either *LMBRD1* or *ABCD4* genes result in impaired release of cobalamin from lysosomes. In the cytoplasm, cobalamin binds to the MMACHC protein (see *cbl*C later), which removes a moiety attached to cobalt in the cobalamin molecule and reduces the cobalt from oxidation state +3 (cob[III]alamin) to +2 (cob[II]alamin). It then enters the mitochondria, where it is catalyzed by MMAB (*cbl*B) and MMAA (*cbl*A) to form adenosylcobalamin, a coenzyme for methylmalonyl-CoA mutase. The other arm of the pathway directs cytosolic cobalamin toward methionine synthase reductase (*cbl*E), which forms methylcobalamin, acting as a coenzyme for methionine synthase (*cbl*G, see Fig. 105.3). The MMADHC protein (see *cbl*D) appears to play a role in determining whether cobalamin enters the mitochondria or remains in the cytoplasm.

The uptake of TCII-Cbl by cells is impaired in individuals with pathogenic variants affecting the transcobalamin receptor *(CD320),* which is located on the cell surface. Individuals homozygous for pathogenic variants in the *CD320* gene encoding the transcobalamin receptor may have mild elevations of methylmalonic acid in the blood and urine. These patients can be identified by the newborn screen based on the elevated propionylcarnitine (C3-carnitine). In **transcobalamin receptor deficiency**, methylmalonic acid levels and plasma propionylcarnitine tend to normalize in the first year of life. It is not clear whether a long-term clinical phenotype is associated with this defect.

Nine different defects in the intracellular metabolism of cobalamin have been identified. These are designated *cbl*A through *cbl*G, *cbl*J, and *cbl*X, where *cbl* stands for a defect in any step of cobalamin metabolism. The *cbl*A, *cbl*B, and *cbl*D-MMA defects cause methylmalonic acidemia *alone*. In patients with *cbl*C, *cbl*D-combined methylmalonic acidemia and homocystinuria, *cbl*F, *cbl*J, and *cbl*X defects, synthesis of both adenosylcobalamin and methylcobalamin is impaired, resulting in *combined* methylmalonic acidemia and homocystinuria. The *cbl*D-homocystinuria, *cbl*E, and *cbl*G defects affect only the synthesis of methylcobalamin, resulting in homocystinuria without methylmalonic aciduria (see Chapter 105.3).

Biochemical manifestations of patients with isolated methylmalonic acidemia caused by *mut*^{0}, *mut*$^-$, *cbl*A, *cbl*B, and *cbl*D-MMA overlap. The wide variations in the severity of the clinical course range from very sick newborn infants to apparently asymptomatic adults. In **severe** forms, lethargy, feeding problems, vomiting, a sepsis-like picture, tachypnea (from metabolic ketoacidosis), and hypotonia may develop in the first few days of life and may progress to hyperammonemic encephalopathy, coma, and death if left untreated. Infants who survive the first attack may go on to develop similar acute metabolic episodes during a catabolic state such as infection or prolonged fasting or after ingestion of a high-protein diet. In certain situations, such acute events can cause a sudden injury of the basal ganglia, a metabolic stroke, resulting in a debilitating movement disorder. Between the acute attacks, the patient usually continues to exhibit hypotonia and feeding problems with failure to thrive, while other complications of the disease occur with age, including recurrent episodes of pancreatitis, bone marrow suppression, osteopenia, and optic nerve atrophy. Chronic renal failure and tubulointerstitial nephritis necessitating renal transplant have been reported in older patients. Renal complications are more severe in patients with the *mut*^{0} and severe *cbl*B forms of methylmalonic acidemia. In milder forms, patients may present later in life with hypotonia, failure to thrive, and developmental delay. Neurocognitive development of patients with mild methylmalonic acidemia may remain within the normal range.

The episodic nature of the condition and its biochemical abnormalities in some patients may be confused with those of *ethylene glycol (antifreeze) ingestion*. Furthermore, the peak of propionate in a blood sample from an infant with methylmalonic acidemia has been mistaken for ethylene glycol when the sample was assayed by gas chromatography without mass spectrometry.

Laboratory findings include ketosis, metabolic acidosis, hyperglycinemia, hyperammonemia, hypoglycemia, anemia, neutropenia, thrombocytopenia, and the presence of large quantities of methylmalonic acid in body fluids (see Fig. 105.6). Metabolites of propionic acid (3-hydroxypropionate and methylcitrate) are also found in the urine. The plasma acylcarnitine profile reveals elevated propionylcarnitine (C3-carnitine) and methylmalonylcarnitine (C4DC-carnitine). Hyperammonemia in methylmalonic acidemia may be confused with a urea cycle disorder. However, patients with defects in urea cycle enzymes are typically *not* acidotic and tend to have high plasma glutamine (see Fig. 105.12). The reason for hyperammonemia is not well understood, but it is likely related to the inhibition of the proximal urea cycle in the mitochondrial matrix.

The diagnosis can be confirmed by identifying pathogenic variants in the causal gene, by measuring propionate incorporation with complementation analysis in cultured fibroblasts, and by measuring the specific activity of the mutase enzyme in biopsies or cell extracts.

Treatment of acute attacks is similar to propionic acidemia. Long-term treatment consists of administration of a low-protein diet limited to the recommended dietary allowance and L-carnitine (50-100 mg/kg/day orally). Patients with severe forms of methylmalonic acidemia may require protein diet modifications similar to those prescribed for patients with propionic acidemia. Patients with isolated methylmalonic acidemia caused by defects in the intracellular metabolism of cobalamin (*cbl*A, *cblD*-MMA, and some patients with *cbl*B) respond to parenteral hydroxocobalamin. Chronic bicarbonate replacement therapy is usually required to correct chronic acidosis. Carglumic acid is used to improve ureagenic function by stimulating its first step catalyzed by the carbamoyl-phosphate synthetase 1 (CPS1) and to facilitate ammonia detoxification during acute hyperammonemia. Ammonia scavengers (sodium benzoate, sodium phenylacetate, sodium phenylbutyrate) should be used cautiously. Plasma ammonia tends to normalize between attacks, and chronic treatment of hyperammonemia is rarely needed. Stressful situations that may trigger acute attacks (infection, prolonged fasting, trauma, surgeries, high-protein meals) should be treated promptly.

Inadequate oral intake secondary to poor appetite, protein overrestriction, or essential amino acid deficiencies is a common complication in the long-term management of these patients. Consequently, enteral feeding through gastrostomy is often recommended early in the course of treatment. Close monitoring of blood pH, essential amino acid levels, blood and urinary concentrations of methylmalonate, and growth parameters is required to ensure that the nutritional prescription meets the patient's metabolic demands. In addition, frequent monitoring of kidney function, vision, hearing, and bone mineral density are necessary for early recognition and management of chronic complications. Glutathione deficiency responsive to treatment with ascorbate has been described.

Liver, kidney, and combined liver-kidney transplantations have been attempted in an increasing number of affected patients. Liver and liver-kidney transplantation can alleviate, but not eliminate, the metabolic abnormalities. Furthermore, liver and liver-kidney transplants do not provide complete protection against the occurrence of metabolic stroke. Kidney transplantation alone can restore the renal function but results in only minor improvement of the clinical stability of patients.

Prognosis depends on the severity of symptoms and the occurrence of complications. In general, patients with complete deficiency of mutase apoenzyme (*mut*^{0}) and severe forms of *cbl*B deficiency have the least favorable prognosis, and those with *mut*$^-$ and *cbl*A defects have a better outcome.

Methylmalonic acidemia can be identified on the universal newborn screening by measuring propionylcarnitine (C3) using tandem mass spectrometry. The prevalence of all forms of methylmalonic aciduria is estimated at 1 in 50,000 to 1 in 100,000 live births. All defects causing isolated methylmalonic acidemia are inherited in an autosomal recessive manner. Pathogenic variants in the genes for *cbl*A *(MMAA)*, *cbl*B *(MMAB)*, and all forms of *cbl*D *(MMADHC)* have been identified in affected patients. The previously described *cbl*H group is identical to the *cbl*D-MMA defect.

COMBINED METHYLMALONIC ACIDURIA AND HOMOCYSTINURIA (*cblC, epi-cblC, cblD, cblF, cblJ,* AND *cblX* DEFECTS)

Combined methylmalonic acidemia and homocystinuria caused by *cbl*C deficiency is the most common type of intracellular cobalamin (vitamin B_{12}) biosynthesis defect. Deficiency of *cbl*C is as common as methylmalonyl-CoA mutase deficiency. The other disorders (*cbl*D, *cbl*F, *cbl*J, *cbl*X) are much rarer (see Figs. 105.3 and 105.4). Neurologic findings are prominent in patients with *cbl*C, *epi-cblC*, *cbl*D-combined, and *cbl*X defects. Most patients with the *cbl*C defect present in the first month of life because of failure to thrive, lethargy, poor feeding, developmental delay, nystagmus, and seizures. Hyperammonemia may be seen infrequently, whereas hyperglycinemia is not present, unlike in isolated *mut*-type methylmalonic acidemia. Intrauterine growth restriction and microcephaly suggest that *cbl*C can manifest prenatally in some affected infants. Late-onset patients with sudden development of dementia and myelopathy have been reported, even with presentation in adulthood. Megaloblastic anemia is a common finding in patients with *cbl*C defect. Mild to moderate increases in concentrations of methylmalonic acid and significant elevations in total plasma homocysteine are found in blood. Unlike classic homocystinuria, in untreated *cbl*C, patients' plasma methionine is low to normal. Retinal abnormalities (e.g., bull's eye maculopathy) resulting in severe progressive vision loss are common and can be seen as early as 3 months of age, even in prospectively identified and well-treated patients. **Thrombotic microangiopathy** can present as hemolytic uremic syndrome, pulmonary hypertension, and cor pulmonale. Hydrocephalus and noncompaction cardiomyopathy have been reported as complications in patients with *cbl*C defect.

Similar to *cblC* patients, males with *cbl*X have elevations of both total plasma homocysteine and methylmalonic acid, but they tend to have milder elevations of these metabolites. Unlike *cbl*C-deficient patients, who tend to respond to treatment, *cbl*X-deficient patients experience failure to thrive, severe developmental delay, and intractable epilepsy despite aggressive treatment.

Clinical findings in *cbl*F deficiency are quite variable. Patients may present with poor feeding, growth and developmental delay, and persistent stomatitis manifesting in the first months of life. Delay in diagnosis and treatment can be accompanied by hyperpigmentation of skin, developmental delay, intellectual disability, and short stature. Vitamin B_{12} malabsorption and low plasma vitamin B_{12} has been noted in patients with *cbl*F defect. Clinical manifestations of *cblJ* defect show significant overlap with those of the *cbl*F deficiency. Dysmorphic features and congenital heart disease have been reported in some patients with *cbl*F and *cbl*J defects.

Experience with **treatment** of patients with *cbl*C, *cbl*D, *cbl*F, *cbl*J, and *cbl*X defects is limited. Large doses of hydroxocobalamin (up to 0.3 mg/kg/day) in conjunction with betaine (up to 250 mg/kg/day) produce biochemical improvement with variable clinical effect. Patients with *cbl*F and *cblJ* deficiency typically show a favorable biochemical and clinical response to smaller hydroxocobalamin doses (1 mg once weekly to 1 mg daily parenterally). Folic or folinic acid supplementation is recommended. Dietary methionine deficiency should be avoided.

The *cbl*C disorder is caused by pathogenic variants in the *MMACHC* gene. A frameshift variant (c.271dupA) is seen in up to 40% of *MMACHC* alleles and is associated with a less favorable clinical outcome. Epi-*cbl*C, with a similar phenotype, is caused by compound heterozygous variants in *MMACHC* and *PRDX1,* a neighboring gene. Pathogenic variants in *PRDX1* cause hypermethylation and silencing of the promoter/exon 1 of *MMACHC,* resulting in repressed gene expression. The *cbl*D disorder is caused by pathogenic variants in the *MMADHC* gene. Pathogenic variants resulting in *cbl*D-homocystinuria affect the C-terminal domain of the gene product; those resulting in *cbl*D-MMA (e.g., causing only methylmalonic aciduria) affect the N-terminus. Patients with classic *cbl*D, with both homocystinuria and methylmalonic acidemia, have pathogenic variants resulting in decreased protein expression. The *cbl*F disorder is caused by pathogenic variants in the *LMBRD1* gene encoding a lysosomal membrane protein. The *cblJ* disorder is associated with pathogenic variants in the *ABCD4*

gene, encoding an adenosine triphosphate–binding cassette protein localized to the lysosomal membrane. The *cbl*X disorder is caused by pathogenic variants in the *HCFC1* gene on the X chromosome (Xq28), which encodes a transcription factor that appears to be essential for expression of the *MMACHC* gene. This is the only X-linked disorder in the B_{12} intracellular metabolism pathway. Rare defects resulting in a phenotype overlapping with *cbl*X deficiency have been associated with biallelic pathogenic variants in the genes *THAP11* or *ZNF143*.

ISOLATED HOMOCYSTINURIA

Patients with *cbl*D variant one, *cbl*E, and *cbl*G deficiency present with isolated homocystinuria without methylmalonic acidemia (see Chapter 105.3).

COMBINED MALONIC AND METHYLMALONIC ACIDURIA (*ACSF3*-RELATED DISORDER)

Combined *m*alonic and *m*ethyl*m*alonic *a*ciduria **(CMAMMA)** is a rare autosomal recessive disorder resulting from pathogenic variants in *ACSF3*. ACSF3 is a putative acyl-CoA synthetase required for the conversion of malonic and methylmalonic acids to their CoA derivatives in the mitochondrial matrix. The disorder can be suspected based on the presence of elevated malonic and methylmalonic acids in urine and plasma. It is distinguished from malonyl-CoA decarboxylase because methylmalonic acid is about fivefold greater than malonic acid in the urine. Plasma propionylcarnitine (C3-carnitine) in CMAMMA patients is normal, so universal newborn screening programs using C3-carnitine in blood spots to screen for methylmalonic acidemia would not detect this condition. The clinical phenotype is incompletely understood. Young patients identified prospectively in infancy through urine-based newborn screening were reported to be asymptomatic, but the long-term outcome in this cohort awaits further characterization. Older patients ascertained clinically have highly variable presentations, including metabolic crises, failure to thrive, seizures, memory problems, optic nerve or spinal cord atrophy, and progressive neurodegeneration. Treatment of CMAMMA is supportive and includes avoidance of an excessively high-protein diet. Vitamin B_{12} supplementation does not appear to lower malonic and methylmalonic metabolites in body fluids.

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105.7 Glycine

Oleg A. Shchelochkov and Charles P. Venditti

Glycine is a nonessential amino acid synthesized by multiple endogenous sources, including serine, choline, and threonine. Structurally, it is the simplest amino acid. Glycine is involved in many reactions in the body, especially in the nervous system, where it functions as a neurotransmitter (excitatory in the cortex, inhibitory in the brainstem and the spinal cord; see Chapter 105.11). Its main catabolic pathway requires the *glycine cleavage system,* a pyridoxal phosphate–dependent, mitochondrial enzyme complex that converts glycine to carbon dioxide and ammonia and transfers α-carbon to tetrahydrofolate (see Fig. 105.8). The glycine cleavage system is composed of four proteins: P protein (glycine decarboxylase), H protein, T protein, and L protein, which are encoded by four different genes.

HYPOGLYCINEMIA

Defects in the biosynthetic pathway of serine (see Chapter 105.8) cause a deficiency of glycine in addition to that of serine in body fluids, especially in the CSF. Isolated primary deficiency of glycine has not been reported.

HYPERGLYCINEMIA

Elevated levels of glycine in body fluids occur in propionic acidemia, methylmalonic acidemia, isovaleric acidemia, and β-ketothiolase deficiency, which are collectively referred to as **ketotic hyperglycinemia** because of the coexistence of acidosis and ketosis. The pathogenesis of hyperglycinemia in these disorders is not understood. Inhibition of the glycine cleavage enzyme system by the various organic acids and post-translational modification has been shown to occur in some of these biochemical disorders. The term **nonketotic hyperglycinemia (NKH)** is reserved for the clinical condition caused by the genetic deficiency of the *glycine cleavage enzyme system* (GCS, see Fig. 105.8). In this condition, hyperglycinemia is present without ketosis.

NONKETOTIC HYPERGLYCINEMIA (GLYCINE ENCEPHALOPATHY)

Four forms of NKH have been identified: neonatal, infantile, late onset, and transient. NKH is an autosomal recessive disorder caused by biallelic pathogenic variants in either of three genes, *GLDC* (encodes P protein), *AMT* (encodes T protein), and *GCSH* (encodes H protein). The L-protein gene *(DLD)* encodes dihydrolipoamide dehydrogenase, the E3 component of α-ketoacid dehydrogenase complexes, and is discussed in Chapter 105.6. Birth prevalence of NKH is ~1:75,000, but a high frequency of the disorder has been noted in Northern Finland (1:12,000 live births), suggesting that this disorder is likely underdiagnosed in some regions of the world.

NKH should be differentiated from the GCS cofactor deficiency caused by the deficiency of lipoate resulting from biallelic pathogenic variants in genes involved in lipoate synthesis (*LIAS, LIPT2, BOLA3, GLRX5, IBA57,* and *NFU1*).

Neonatal Nonketotic Hyperglycinemia

This is the most common form of NKH. Clinical manifestations become apparent in the first few days of life (between 6 hours and 8 days after birth). Poor feeding, failure to suck, lethargy, and profound hypotonia may progress rapidly to a deep coma, apnea, and death. Convulsions, especially myoclonic seizures, and hiccups are common.

Laboratory findings reveal moderate to severe hyperglycinemia (as high as eight times the upper reference range) and hyperglycinuria. The unequivocal elevation of glycine concentration in CSF (15-30 times the upper reference range) and the high ratio of glycine concentration in CSF to that in plasma (a value >0.08, reference value <0.02) are diagnostic of NKH. Affected patients' blood pH is usually normal. The plasma acylcarnitine profile and urine organic acid assay reveal no abnormalities. CSF serine levels can be low.

Approximately 30% of NKH infants die despite supportive therapy. Those who survive develop profound intellectual disability and intractable seizure disorders (myoclonic and/or grand mal seizures). Hydrocephalus, requiring shunting, and pulmonary hypertension have been noted in survivors. Transient hyperglycemia may prompt evaluation for biochemical phenocopies of NKH (e.g., organic acidemias), brain imaging studies to evaluate for intracerebral hemorrhage or hypoxic-ischemic injury, and ultimately may require molecular studies of *AMT, GLDC,* and *GCSH*.

Transient Nonketotic Hyperglycinemia

Most clinical and laboratory manifestations of transient NKH are indistinguishable from those of the neonatal form. By 2-8 weeks of age, however, a complete clinical recovery may occur, and the elevated glycine levels in plasma and CSF normalize after the patient stops a glycine-lowering medication. Some of these patients develop normally with no neurologic sequelae, but intellectual disability has been noted in others. The etiology of this condition is not known, but it is thought to be a consequence of immaturity of the enzyme system; genetic testing is normal. Transient hyperglycemia should prompt consideration of additional biochemical studies; brain imaging; and molecular studies to evaluate for pathogenic variants in *AMT, GLDC,* and *GCSH*.

Infantile Nonketotic Hyperglycinemia

In infantile NKH, previously healthy-appearing infants develop signs and symptoms of neonatal NKH after 6 months of age. Seizures and hypotonia are common presenting signs. Infantile NKH appears to be a milder form of neonatal NKH; infants usually survive, and intellectual

disability is not as profound as in the neonatal form. Laboratory findings in patients with infantile NKH are identical to those seen in neonatal NKH.

Late-Onset Nonketotic Hyperglycinemia

Clinical manifestations of this atypical form of NKH include progressive spastic diplegia, optic nerve atrophy, and choreoathetotic movements. Age of onset has been between 2 and 33 years. Symptoms of delirium, chorea, and vertical gaze palsy may occur episodically in some patients during an intercurrent infection. Mental development is usually normal, but mild cognitive impairment and infrequent seizures have been reported in some patients.

Laboratory findings in late-onset NKH are similar but not as pronounced as in neonatal NKH.

All forms of NKH should be differentiated from *ketotic* hyperglycinemia, pyridox(am)ine phosphate oxidase (PNPO) deficiency, ingestion of valproic acid, and transient glycine encephalopathy. Valproic acid can moderately increase blood, CSF, and urinary concentrations of glycine. Repeat assays after discontinuation of the drug will help establish the diagnosis.

Diagnosis and Treatment

A diagnosis of NKH can be suspected based on the findings of elevated glycine in plasma or CSF and the abnormal CSF/plasma ratio of glycine. The diagnosis is confirmed using molecular analysis of the NKH-related genes (*AMT, GLDC,* and *GCSH*). Rarely, enzymatic assay on liver specimens is necessary to establish the diagnosis. Enzyme activity in the neonatal form is close to zero, whereas in the other forms, some residual activity is present. In most patients with neonatal NKH, the enzyme defect resides in the P protein (75%). Defects in the T protein account for approximately 20% of cases, whereas <1% are caused by pathogenic variants in the H protein.

Prenatal diagnosis can be accomplished by identifying known familial pathogenic variants in the affected gene or by performing an assay of the enzyme activity in chorionic villus biopsy specimens.

No effective treatment is currently available. Exchange transfusion, dietary restriction of glycine, and administration of sodium benzoate or folate have not altered the neurologic outcome in severe forms of NKH. Patients with attenuated NKH may experience clinical improvement from enteral sodium benzoate. Drugs that counteract the effect of glycine on neuronal cells, such as dextromethorphan and felbamate, have shown some beneficial effects in patients with the mild forms of the condition.

SARCOSINEMIA

Increased concentrations of sarcosine (*N*-methylglycine) are observed in both blood and urine of probands affected by sarcosine dehydrogenase complex deficiency. This autosomal recessive metabolic condition is caused by a defect in sarcosine dehydrogenase, the enzyme that converts sarcosine to glycine (see Fig. 105.8) and is encoded by *SARDH*. No consistent clinical picture has been attributed to sarcosinemia.

PRIMARY TRIMETHYLAMINURIA

Trimethylamine is normally produced by intestinal bacteria from the breakdown of dietary choline and trimethylamine oxide by bacteria. Egg yolk and liver are the main sources of choline, and fish is the major source of trimethylamine oxide. Trimethylamine is absorbed and oxidized in the liver by trimethylamine oxidase (a flavin-containing monooxygenase encoded by *FMO3*) to trimethylamine oxide, which is odorless and excreted in the urine (see Fig. 105.8). Deficiency of this enzyme results in massive excretion of trimethylamine in urine. There is a body odor that resembles that of rotting fish, which may have significant psychosocial ramifications. Transient symptomatic trimethylaminuria can occur in normal individuals after ingestion of large quantities of the previously mentioned foods.

Treatment with oral activated charcoal and short courses of oral metronidazole, neomycin, or lactulose cause temporary reduction in body odor. Restriction of fish, eggs, liver, and other sources of choline (e.g., nuts, grains) in the diet significantly reduces the odor. Topical use of acidic soaps (pH 5.5) can also help control the odor.

HYPEROXALURIA AND OXALOSIS

Normally, oxalic acid is derived mostly from oxidation of glyoxylic acid and, to a lesser degree, from oxidation of ascorbic acid (see Fig. 105.8). Glyoxylic acid is formed from oxidation of glycolic acid and glycine in the peroxisomes and catabolism of **hydroxyproline** in the mitochondria mediated by 4-hydroxy-2-oxoglutarate aldolase 1 (encoded by *HOGA1*), the underlying cause of **primary hyperoxaluria type 3** (Fig. 105.9). Vegetables and foods containing oxalic acid, such as spinach, rhubarb, and almond milk, are the main *exogenous* sources of glycolic and oxalic acids; most of glyoxylic and oxalic acids are produced endogenously. Normally, a major portion of glyoxylate produced in the body is shuttled to peroxisomes, where it is converted to glycine by the action of the enzyme alanine:glyoxylate transaminase (AGT encoded by *AGXT*). Deficiency of this enzyme causes **primary hyperoxaluria** type 1. Most of the remaining glyoxylate in the cytosol is reduced to glycolate by the action of the enzyme glyoxylate reductase/

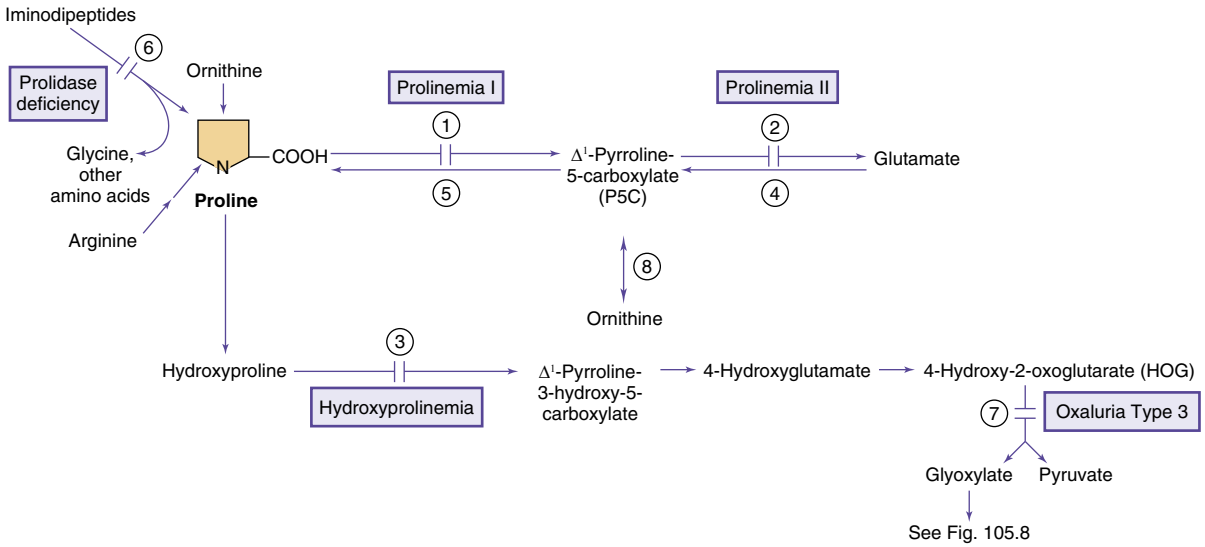

Fig. 105.9 Pathways in the metabolism of proline. **Enzymes:** *(1)* Proline oxidase (dehydrogenase), *(2)* Δ^1-pyrroline-5-carboxylic acid (P5C) dehydrogenase, *(3)* hydroxyproline oxidase, *(4)* Δ^1-pyrroline-5-carboxylic acid (P5C) synthase, *(5)* Δ^1-pyrroline-5-carboxylic acid (P5C) reductase, *(6)* prolidase, *(7)* 4-hydroxyoxoglutarate aldolase one (HOGA1), *(8)* ornithine aminotransferase.

hydroxypyruvate reductase (GR/HPR encoded by *GRHPR*). Deficiency of this enzyme causes **primary hyperoxaluria type 2**. These pathways protect the body from excessive production of oxalic acid (see Fig. 105.8). Any glyoxylate that cannot be disposed of through these pathways is readily converted to oxalic acid by the action of the enzyme lactate dehydrogenase (LDH). Oxalic acid cannot be further metabolized in humans and is excreted in the urine as oxalates. Calcium oxalate is relatively insoluble in water and precipitates in tissues (kidneys and joints) if its concentration increases in the body.

Secondary hyperoxaluria has been observed in pyridoxine deficiency (cofactor for alanine:glyoxylate transaminase), in patients with IBD, extensive resection of the small bowel or jejunoileal bypass *(enteric hyperoxaluria)*, after ingestion of ethylene glycol or high doses of vitamin C, and after administration of the anesthetic agent methoxyflurane (which can be catabolized to oxalic acid as one of its by-products). Acute, fatal hyperoxaluria may develop after ingestion of plants with high oxalic acid content (e.g., sorrel) or intentional ingestion of oxalic acid. Precipitation of calcium oxalate in tissues causes hypocalcemia, liver necrosis, renal failure, cardiac arrhythmia, and death. The lethal dose of oxalic acid is estimated at 5-30 g.

Primary hyperoxaluria is a group of disorders in which large amounts of oxalates accumulate in the body. Three types of primary hyperoxaluria have been identified to date. The term **oxalosis** refers to deposition of calcium oxalate in parenchymal tissues.

Primary Hyperoxaluria Type 1

This rare autosomal recessive condition (prevalence of 1 in 120,000 live births in Europe) is the most common form of primary hyperoxaluria and can be seen in in ~1% of children diagnosed with end-stage renal disease. Primary hyperoxaluria type 1 is caused by deficiency of the peroxisomal enzyme alanine:glyoxylate transaminase *(AGXT)*, which is expressed almost exclusively in the liver peroxisomes and requires pyridoxine (vitamin B_6) as a cofactor. In the absence of this enzyme, glyoxylic acid cannot be converted to glycine and is transferred to the cytosol, where it is oxidized to oxalic acid (see earlier and Fig. 105.8).

The age of presentation varies widely, from the neonatal period to late adulthood. The majority of patients become symptomatic in late childhood or early adolescence. In about 20% of cases, symptoms develop before the infant's first birthday. The initial clinical manifestations are related to renal stones and nephrocalcinosis. Renal colic and asymptomatic hematuria lead to a gradual deterioration of renal function, manifested by growth retardation and uremia. If the disorder is left untreated, most patients die before 20 years of age from renal failure. Other frequent manifestations of the disease include failure to thrive, short stature, arterial calcifications, arrhythmia, heart failure, hypothyroidism, and skin nodules. Acute arthritis is a rare manifestation and can be misdiagnosed as gout because uric acid is often elevated in patients with type 1 hyperoxaluria, driven in part by the worsening renal function. Crystalline retinopathy and optic neuropathy causing visual loss have been reported.

A marked increase in urinary excretion of oxalate (typical excretion: 10-50 mg/day) is the most important laboratory finding. The presence of oxalate crystals in urinary sediment is rarely helpful for diagnosis because such crystals can also be seen in otherwise healthy individuals. Urinary excretion of glycolic acid and glyoxylic acid is increased in most, but not all, patients. Diagnosis can be confirmed by identification of biallelic pathogenic variants in the *AGXT* gene or by performing an enzymatic assay in liver specimens.

The **diagnosis** of primary hyperoxaluria type 1 can be suspected in patients presenting with recurrent renal stones, nephrocalcinosis, and oxalate crystals in the urine after ruling out possible secondary causes (e.g., gastrointestinal disorders or dietary causes). Laboratory studies will reveal elevated urinary oxalate excretion, high urinary glycolate, and elevated plasma oxalate. Confirmatory testing of *AGXT* as a single gene or part of a multigene panel will secure the ultimate diagnosis. The most common pathogenic variant in patients with high residual enzyme activity (c.508G>A, p.Gly170Arg) results in mislocalization of the enzyme to mitochondria instead of peroxisomes, thus leading to the loss of in vivo function. **Prenatal diagnosis** has been achieved by DNA analysis of chorionic villus samples when biallelic pathogenic variants are known.

Treatment focuses on the reduction of oxalic acid production and on improving calcium oxalate disposal. Patients with primary hyperoxaluria type 1 should receive a 3-month trial of pyridoxine treatment to establish pyridoxine responsiveness. In up to 30% of patients (e.g., those homozygous for the *AGXT* pathogenic variant c.508G>A), administration of large doses of pyridoxine can reduce the plasma level and urinary excretion of oxalate. To increase calcium oxalate disposal and prevent nephrolithiasis, high oral fluid intake (2-3 L/m^2/day while controlling for fluid balance), urine alkalinization, phosphate supplementation, monitoring of vitamin C and vitamin D intake, and avoidance of drugs that can increase urinary calcium excretion (e.g., loop diuretics) are recommended. Urinary stones should be managed by experienced urologists, as excessive surgical trauma may contribute to renal dysfunction. Renal function replacement strategies (e.g., hemodialysis) are used in some patients (e.g., to bridge patients to transplant or when transplant is not a viable option).

Organ **transplantation** has emerged as the most definitive treatment. The decision to undergo kidney, liver, or liver-kidney transplant is complex, and referral rates may vary from one medical center to another. Except for older patients with the pyridoxine-responsive form of disease, renal transplantation alone in patients with renal failure may not improve the outcome, because oxalosis can recur in the transplanted kidney. Combined liver-kidney transplants have resulted in a significant decrease in plasma and urinary oxalate and thus may be the most effective treatment strategy, particularly in children.

Primary Hyperoxaluria type 2 (L-Glyceric Aciduria)

This rare autosomal recessive condition is caused by a deficiency of the glyoxylate reductase–hydroxypyruvate reductase enzyme complex encoded by *GRHPR* (see Fig. 105.8). A deficiency in the activity of this complex results in accumulation of two intermediate metabolites: hydroxypyruvate (the ketoacid derivative of serine) and glyoxylic acid. Both these compounds are further metabolized by LDH to L-glycerate and oxalate, respectively. A high prevalence of this disorder is reported in the Saulteaux-Ojibway Indians of Manitoba.

Primary hyperoxaluria type 2 results in the deposition of calcium oxalate in the renal parenchyma and urinary tract. Renal stones presenting with renal colic and hematuria may develop before age 2 years. Renal failure is less common in this condition than in primary hyperoxaluria type 1.

Urinary testing reveals large amounts of L-glyceric acid in addition to high levels of oxalate. Urinary L-glyceric acid is considered a pathognomonic finding in primary hyperoxaluria type 2. Urinary excretion of glycolic acid and glyoxylic acid is not increased. The presence of L-glyceric acid without increased levels of glycolic and glyoxylic acids in urine differentiates this type from type 1 hyperoxaluria. The diagnosis can be confirmed by molecular analysis of *GRHPR* or by the enzyme assay in liver biopsy.

The principles of therapy are similar to those in primary hyperoxaluria type 1. Renal transplant is used in some patients; experience with kidney-liver transplantation is limited at this time.

Primary Hyperoxaluria Type 3

Approximately 10% of patients with primary hyperoxaluria have deficiency of 4-hydroxy-2-oxoglutarate aldolase 1 (HOGA1), the underlying cause of hyperoxaluria type 3. The enzyme is encoded by *HOGA1*. This mitochondrial enzyme catalyzes a step in the metabolic pathway of hydroxyproline that generates pyruvate and glyoxylate from 4-hydroxy-2-oxoglutarate (HOG; see Figs. 105.8 and 105.9). In vitro studies show inhibition of glyoxylate reductase–hydroxypyruvate reductase enzyme activity by a high concentration of HOG that accumulates in patients with hyperoxaluria type 3. HOGA1 deficiency results in a biochemical phenotype similar to primary hyperoxaluria type 2 (see Fig. 105.8).

Patients with primary hyperoxaluria type 3 usually present with calcium oxalate kidney stones in early childhood, but asymptomatic older siblings have also been identified. Gradually, renal function may

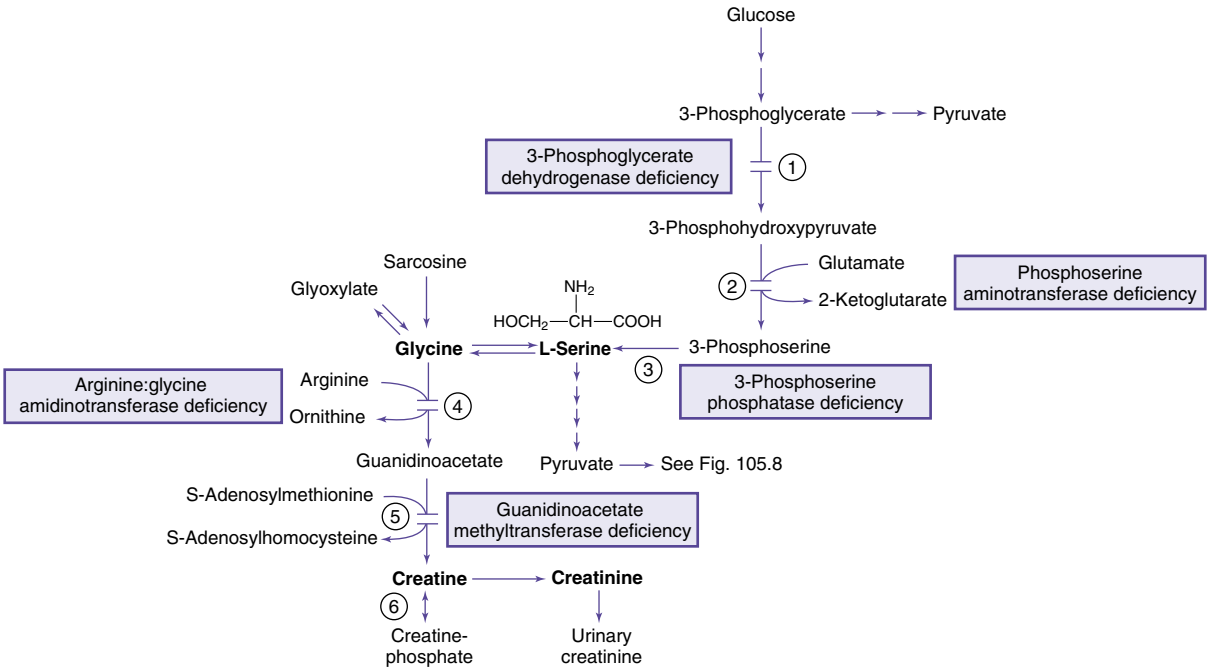

Fig. 105.10 Biosynthesis of serine and creatine. **Enzymes:** *(1)* 3-Phosphoglycerate dehydrogenase, *(2)* 3-phosphoserine aminotransferase, *(3)* 3-phosphoserine phosphatase, *(4)* arginine:glycine amidinotransferase (AGAT), *(5)* guanidinoacetate methyltransferase (GAMT), *(6)* creatine kinase.

decline, infrequently resulting in end-stage renal disease. Increased levels of HOG in urine, serum, and liver biopsy samples of these patients are the distinguishing feature of this disorder. **Treatment** involves high oral fluid intake, management of oral citrate or phosphate intake to prevent calcium oxalate renal stone formation, and avoidance of dehydration to prevent acute kidney injury. In severe forms of this disorder, dialysis and transplantation may be required to address the end-stage renal disease.

Creatine Deficiency Disorders

Creatine is synthesized mainly in the liver, pancreas, and kidneys and to a lesser degree in the brain from arginine and glycine and is transported to muscles and the brain, where there is high activity of the enzyme creatine kinase (Fig. 105.10). Phosphorylation and dephosphorylation of creatine in conjunction with adenosine triphosphate and diphosphate provide high-energy phosphate transfer reactions in these organs. Creatine is nonenzymatically metabolized to creatinine at a relatively constant daily rate and is excreted in the urine. Three genetic conditions are known to cause creatine deficiency in the brain and other tissues. Two enzymes, arginine:glycine amidinotransferase (see Fig. 105.10; **AGAT**, encoded by *GATM*) and guanidinoacetate methyltransferase (**GAMT**, encoded by *GAMT*), are involved in the biosynthesis of creatine. Both conditions may respond to creatine supplementation, especially when the treatment is started at an early age. The third condition, an X-linked inherited defect, is caused by a deficiency of the **creatinine transporter** (CRTR, encoded by *SLC6A8*) mediating uptake of creatine by the brain and muscle. A CRTR defect is the most common cause of creatine deficiency, accounting for up to 1–2% of males with intellectual disability of unknown cause.

Clinical manifestations of the three defects overlap, relate to the brain and muscle, and may appear in the first few weeks or months of life. Developmental delay, intellectual disability, speech delay, psychiatric symptoms (autism and psychosis), hypotonia, ataxia, and seizures are common findings. Dystonic movements have been documented in GAMT and CRTR deficiency.

Laboratory findings include decreased creatine in plasma in patients with AGAT and GAMT defects. Plasma creatinine level alone is insufficient to diagnose these disorders. Secondary to impaired reabsorption of creatine in kidneys, the urinary ratio of creatine to creatinine is increased in male patients with a CRTR defect but can also be mildly elevated in female carriers. Marked elevations of guanidinoacetate in the blood, urine, and especially in CSF are diagnostic of GAMT defects. In contrast, low levels of guanidinoacetate can be found in body fluids in the AGAT defect. Evidence of creatine and creatine phosphate deficiency (in all three defects) and high levels of guanidinoacetate (in the GAMT defect) in the brain can be demonstrated by magnetic resonance spectroscopy (MRS). Brain MRI may show signal hyperintensity in the globus pallidus. Diagnosis of AGAT deficiency or GAMT deficiency may be confirmed by DNA analysis or by measuring enzymatic activity in cultured fibroblasts (GAMT) or lymphoblasts (AGAT). The diagnosis of CRTR deficiency can be confirmed by DNA analysis or a creatine uptake assay in fibroblasts.

The outcomes of **treatment** are age-dependent, and the best outcomes are seen when treatment is started in the neonatal period or presymptomatically. In AGAT-deficient patients, oral creatine monohydrate (up to 400-800 mg/kg/day) can improve muscle weakness, seizures, and neurocognitive outcomes in some patients. In GAMT-deficient patients, supplementation with oral creatine monohydrate (up to 400-800 mg/kg/day), ornithine (up to 400-800 mg/kg/day), and dietary arginine restriction may result in improved muscle tone and neurocognitive development and may alleviate seizures. In CRTR-deficient patients, administration of creatine monohydrate and its precursors (arginine and glycine) may improve seizures and neurocognitive outcomes in some patients.

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105.8 Serine Deficiency Disorders (Serine Biosynthesis and Transport Defects)

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Serine is a nonessential amino acid supplied through dietary sources and through endogenous synthesis, mainly from glucose and glycine. The endogenous production of serine comprises an important portion of the daily requirement of this amino acid, especially in the synaptic junctions, where it contributes to phospholipid metabolism of D-serine and glycine, both of which are involved in neurotransmission (see Chapter 105.11). Consequently, deficiency of any of the enzymes

involved in the biosynthesis of serine or its transport causes neurologic manifestations. The clinical spectrum of serine deficiency disorders ranges widely and varies from **Neu-Laxova syndrome** on the severe end of spectrum to epilepsy and developmental delay on the milder end. Affected patients respond favorably to oral supplementation with serine and glycine when treatment is initiated very early in life. Figures 105.8 and 105.10 show the metabolic pathway for the synthesis and catabolism of serine.

3-PHOSPHOGLYCERATE DEHYDROGENASE DEFICIENCY

3-Phosphoglycerate dehydrogenase (**PHGDH** encoded by *PHGDH*) deficiency has a broad range of symptoms and ages of presentation. **Neu-Laxova syndrome type 1**, an autosomal recessive condition, is on the most severe end of the spectrum, presenting prenatally with intrauterine growth restriction and congenital anomalies, including dysmorphic facial features, microcephaly, CNS malformations, limb deformities, and ichthyosis. Most patients with this form are stillborn or have early neonatal mortality. The infantile form of PHGDH deficiency can present with feeding problems, failure to thrive, vomiting, irritability, seizures, severe developmental delay, and hypertonia progressing to spastic quadriplegia. Nystagmus, cataracts, hypogonadism, and megaloblastic anemia have been observed in some affected infants. Patients with a milder form of this disorder experience cognitive impairment, behavioral problems, sensorineural polyneuropathy, and childhood-onset seizures.

Laboratory findings include low fasting levels of serine and glycine in plasma and very low levels of serine and glycine in CSF. No abnormal organic acid metabolite is found in the urine. MRI of the brain shows cerebral atrophy with enlarged ventricles, significant attenuation of white matter, and impaired myelination. **Diagnosis** can be confirmed by DNA analysis or by measurement of the enzyme activity in cultured fibroblasts. **Treatment** with high doses of serine (200-700 mg/kg/day orally) and glycine (200-300 mg/kg/day) normalizes the serine levels in the blood and CSF. When started postnatally, this treatment may improve seizures, spasticity, and brain myelination. One case report suggests that developmental delay may be prevented if the treatment commences in the first days of life or prenatally.

If familial pathogenic variants are known, molecular **prenatal diagnosis** is possible. Administration of serine to a mother carrying an affected fetus was associated with stabilization of the fetal head circumference, as evidenced by ultrasound. Treatment with supplemental serine continued postnatally, and the patient remained normal neurologically at 4 years of age. The favorable response of this condition to a relatively straightforward treatment makes this diagnosis an important consideration in any child with microcephaly and neurologic defects such as psychomotor delay or a seizure disorder. Measurements of serine and glycine in the CSF are critical for diagnosis because mild decreases of these amino acids in the plasma can be easily overlooked.

PHOSPHOSERINE AMINOTRANSFERASE DEFICIENCY

Phosphoserine aminotransferase 1 (**PSAT1** encoded by *PSAT1*) catalyzes conversion of 3-phosphohydroxypyruvate to 3-phosphoserine (see Fig. 105.10). Deficiency of this enzyme, an autosomal recessive disorder, may present in the neonatal period with poor feeding, cyanotic episodes, and irritability and may progress to intractable, multifocal seizures and microcephaly. Brain imaging may reveal generalized cerebral and cerebellar atrophy. Laboratory studies done on postprandial plasma samples may reveal normal or mildly decreased levels of serine and glycine. Serine and glycine levels are usually more depressed on the CSF amino acid analysis. **Treatment** with serine and glycine as outlined earlier may result in clinical improvement.

3-PHOSPHOSERINE PHOSPHATASE DEFICIENCY

3-Phosphoserine phosphatase catalyzes the final step in the L-serine synthesis, converting 3-phosphoserine to L-serine. Deficiency of this enzyme results in an autosomal recessive disorder with clinical and biochemical findings indistinguishable from the PHGDH and PSAT1 deficiencies. The disorder is caused by biallelic pathogenic variants in *PSPH*.

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105.9 Proline

Oleg A. Shchelochkov and Charles P. Venditti

Proline is a nonessential amino acid synthesized endogenously from glutamic acid, ornithine, and arginine (see Fig. 105.9). Proline and hydroxyproline are found in high concentrations in collagen. Normally, neither of these amino acids is found in large quantities in urine. Excretion of proline and hydroxyproline as *iminopeptides* (dipeptides and tripeptides containing proline or hydroxyproline) is increased in disorders of accelerated collagen turnover, such as rickets or hyperparathyroidism. Proline is also found in synapses, where it can interact with glycine and glutamate receptors (see Chapter 105.11). The catabolic pathway of proline and hydroxyproline produces glyoxylic acid, which can be further metabolized to glycine or oxalic acid (see Fig. 105.8).

Accumulation of proline in tissues is associated with disorders of hyperprolinemia type 1 and hyperprolinemia type 2. Two types of primary hyperprolinemia have been described. Reduced de novo synthesis of proline may manifest with **cutis laxa** (see Fig. 700.8) with **progeroid features** or **spastic paraplegia**.

HYPERPROLINEMIA TYPE I

This rare autosomal recessive condition is caused by a deficiency of proline oxidase (proline dehydrogenase; see Fig. 105.9). Most patients with hyperprolinemia type 1 appear asymptomatic, although some may present with intellectual disability, seizures, and behavioral problems. Hyperprolinemia may also be a risk factor for autism spectrum disorders and schizophrenia. The nature of such a wide phenotypic range in this biochemical condition is incompletely understood. The gene encoding proline oxidase *(PRODH)* is mapped to 22q11.2 within the critical region for velocardiofacial syndrome. Laboratory studies reveal high concentrations of proline in plasma, urine, and CSF. Increased urinary excretion of hydroxyproline and glycine is also present, which could be related to saturation of the shared tubular reabsorption mechanism due to massive prolinuria.

No effective treatment has yet emerged. Restriction of dietary proline causes a modest improvement in plasma proline but with no proven clinical benefit.

HYPERPROLINEMIA TYPE II

This is a rare autosomal recessive condition caused by the deficiency of Δ^1-pyrroline-5-carboxylate dehydrogenase (aldehyde dehydrogenase 4, *ALDH4A1*; see Fig. 105.9). Intellectual disability and seizures (usually precipitated by an intercurrent infection) have been reported in affected children, but asymptomatic patients have also been described. The cause for such disparate clinical outcomes is incompletely understood.

Laboratory studies reveal increased concentrations of proline and Δ^1-pyrroline-5-carboxylate (P5C) in blood, urine, and CSF. The presence of P5C differentiates this condition from hyperprolinemia type I. An increased level of P5C in body fluids, especially in the CNS, appears to antagonize vitamin B_6 and leads to vitamin B_6 dependency (see Chapter 105.14). Vitamin B_6 dependency may be the main cause of seizures and neurologic findings in this condition and can explain the variability in clinical manifestations in different patients. **Treatment** with high doses of vitamin B_6 is recommended.

PROLIDASE DEFICIENCY

During collagen degradation, imidodipeptides are formed and are normally cleaved by tissue prolidase. Prolidase deficiency, an autosomal recessive condition caused by biallelic pathogenic variants in *PEPD,* results in the accumulation of imidodipeptides in body fluids. The age at onset varies from 6 months to the third decade of life.

Clinical manifestations vary and include recurrent, severe, and painful skin ulcers typically found on the hands and legs. Other skin lesions may precede ulcers by several years and may include a scaly erythematous maculopapular rash, purpura, and telangiectasia. Most ulcers become infected. Healing of the ulcers may take months. Other findings include developmental delays, intellectual disability, organomegaly, anemia, thrombocytopenia, and immune dysfunction resulting in increased susceptibility to infections (recurrent otitis media, sinusitis, respiratory infection, splenomegaly). Some patients have craniofacial abnormalities such as ptosis, ocular proptosis, hypertelorism, small beaked nose, and prominent cranial sutures. Asymptomatic cases have also been reported. Increased incidence of systemic lupus erythematosus has been noted in children. High levels of urinary excretion of imidodipeptides are diagnostic. The diagnosis can be confirmed using DNA analysis. Enzyme assay may be performed in erythrocytes or cultured skin fibroblasts.

Treatment of prolidase deficiency is supportive. Infectious complications can be fatal and warrant close and proactive antibiotic management. Oral supplementation with proline, ascorbic acid, and manganese and topical proline and glycine have not been found to be consistently effective in all patients.

DISORDERS OF DE NOVO PROLINE SYNTHESIS

De novo synthesis of proline and ornithine from glutamate appears to be critical in the normal biology of connective tissue and to maintain the urea cycle in a repleted state. Correspondingly, clinical manifestations of these disorders encompass connective tissue abnormalities, nervous system abnormalities, and variable biochemical abnormalities reflecting urea cycle dysfunction. Clinical and laboratory findings associated with the deficient function of Δ^1-P5C synthase (see Fig. 105.9), encoded by *ALDH18A1,* and P5C, reductase encoded by *PYCR1,* are discussed.

Deficient activity of P5C synthase has been associated with several phenotypes, including **de Barsy syndrome**, characterized by cataracts, growth restriction, intellectual disability, a prematurely aged appearance (progeroid features), and cutis laxa. Some patients may show pyramidal signs. Skin biopsy may reveal decreased size of elastic fibers and collagen abnormalities. Brain imaging studies show cortical atrophy, ventriculomegaly, and reduced creatine. Laboratory findings include reduced levels of proline, ornithine, citrulline, and arginine as well as mild fasting hyperammonemia. Patients may show only intermittent abnormalities on the plasma amino acid profile, likely related to the time of blood sampling in relation to the last meal. Interestingly, both autosomal recessive and autosomal dominant forms of inheritance have been described. The diagnosis can be suspected in a patient presenting with cutis laxa, developmental delay, mild hyperammonemia, and characteristic amino acid abnormalities. The diagnosis can be confirmed using molecular DNA analysis or the glutamine loading test on skin fibroblasts. Treatment is supportive, although supplementation with citrulline or arginine to address hyperammonemia and cerebral creatine depletion have been proposed.

Biallelic pathogenic variants in *PYCR1* result in the abnormal function of the mitochondrial Δ^1-pyrroline-5-carboxylate reductase, which catalyzes the last step in the synthesis of proline from P5C. The most consistent finding in patients carrying proven pathogenic variants in *PYCR1* include triangular facies, cutis laxa **(de Barsy–like syndrome)**, joint hypermobility, wrinkled skin, gerodermia osteodysplastica, and progeroid features. Skin biopsy reveals reduction of the elastic fibers and infiltration with inflammatory cells. Some patients may have epilepsy, developmental delays, intellectual disability, cataracts, osteopenia, and failure to thrive. However, many of the affected families are consanguineous, thus complicating interpretation of the phenotype. Of note, plasma amino acid analysis reveals no specific abnormalities. The diagnosis depends on the recognition of the skin findings and can be confirmed using molecular DNA analysis. Available pedigrees of families affected by *PYCR1*-related disorder supports the autosomal recessive mode of inheritance.

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105.10 Glutamic Acid

Oleg A. Shchelochkov and Charles P. Venditti

Glutamic acid and its amide derivative glutamine have a wide range of functions in the body. *Glutamate* plays numerous biologic roles, functioning as a neurotransmitter, an intermediate compound in many fundamental biochemical reactions, and a precursor of an inhibitory neurotransmitter, γ-aminobutyric acid (GABA) (see Chapter 105.11). Another major product of glutamate is *glutathione* (γ-glutamylcysteinylglycine). This ubiquitous tripeptide, with its function as the major antioxidant in the body, is synthesized and degraded through a complex cycle called the γ-glutamyl cycle (Fig. 105.11). Because of its free sulfhydryl (−SH) group and its abundance in the cell, glutathione protects other sulfhydryl-containing compounds (e.g., enzymes, coenzyme A) from oxidation. It is also involved in the detoxification of peroxides, including hydrogen peroxide, and in keeping the intracellular milieu in a reduced state. In addition, glutathione participates in amino acid transport across the cell membrane through the γ-glutamyl cycle.

One of the biochemical manifestations of γ-glutamyl cycle deficiency is increased urinary excretion of 5-oxoproline, which could be the result of both genetic and nongenetic causes. 5-Oxoprolinemia should be routinely considered in the differential diagnosis of **high–anion gap metabolic acidosis** (HAGMA). Two metabolic disorders can present with massive 5-oxoprolinuria: **glutathione synthetase deficiency** and **5-oxoprolinase deficiency** (see Fig. 105.11). However, a more common clinical scenario is a transient and mild urinary elevation of 5-oxoproline that can be seen in a variety of metabolic and acquired conditions, such as exposure to acetaminophen and some hydrolyzed-protein formulas, severe burns, Stevens-Johnson syndrome, homocystinuria, urea cycle defects, and tyrosinemia type I.

GLUTATHIONE SYNTHETASE DEFICIENCY

Three forms of this rare autosomal recessive condition have been reported. In the **mild form**, glutathione synthetase deficiency causes glutathione deficiency in erythrocytes. These patients present with hemolytic anemia without chronic metabolic acidosis and demonstrate high residual activity of glutathione synthetase on enzymatic testing. A **moderate form** has also been observed in which the hemolytic anemia is associated with variable degrees of metabolic acidosis and 5-oxoprolinuria. Its **severe form** is distinguished by the presence of hemolytic anemia accompanied by severe acidosis, massive 5-oxoprolinuria, and neurologic manifestations.

Glutathione Synthetase Deficiency, Moderate and Severe Forms

Affected newborn infants with severe and moderate forms of glutathione synthetase deficiency usually develop acute symptoms of metabolic acidosis, jaundice, and mild to moderate hemolytic anemia in the first days of life. Chronic acidosis continues after recovery. Similar episodes of life-threatening acidosis may occur during an infection (e.g., gastroenteritis) or after a surgical procedure. Progressive neurologic damage develops with age, manifested by intellectual disability, spastic tetraparesis, ataxia, tremor, dysarthria, and seizures. Susceptibility to infections, presumably because of granulocyte dysfunction, is observed in some patients. Patients with the moderate form of glutathione synthetase deficiency have milder acidosis and less 5-oxoprolinuria than is seen in the severe form, with few neurologic manifestations.

Laboratory findings include metabolic acidosis, mild to moderate degrees of hemolytic anemia, and 5-oxoprolinuria. High concentrations of 5-oxoproline are also found in the blood. The urinary and blood levels of 5-oxoproline are less pronounced in patients with the moderate form of the condition. The glutathione content of erythrocytes is markedly decreased. Increased synthesis of 5-oxoproline in this disorder is thought to be the result of the conversion of γ-glutamylcysteine to 5-oxoproline by the enzyme γ-glutamyl cyclotransferase (see Fig. 105.11). γ-Glutamylcysteine production increases greatly because the normal inhibitory effect of glutathione on the γ-glutamylcysteine synthetase enzyme is removed.

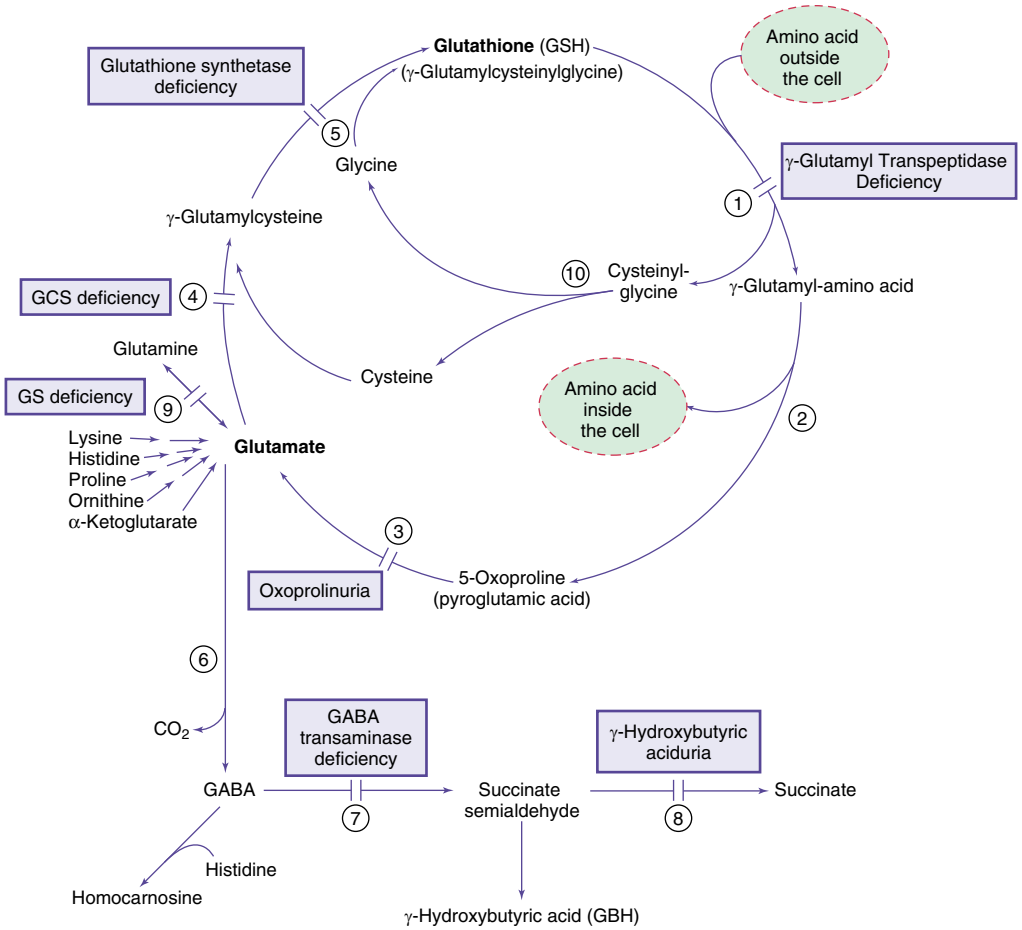

Fig. 105.11 The γ-glutamyl cycle and related pathways. Defects of the glutathione (GSH) synthesis and degradation are noted. **Enzymes:** *(1)* γ-Glutamyl transpeptidase (GGT), *(2)* γ-glutamyl cyclotransferase, *(3)* 5-oxoprolinase, *(4)* γ-glutamyl-cysteine synthetase, *(5)* glutathione synthetase, *(6)* glutamate decarboxylase, *(7)* γ-aminobutyric acid (GABA) transaminase, *(8)* succinate-semialdehyde dehydrogenase, *(9)* glutamine synthetase, *(10)* dipeptidase.

Treatment of acute attack includes hydration, correction of acidosis (by infusion of sodium bicarbonate), and measures to correct anemia and hyperbilirubinemia. Chronic administration of alkali is usually needed indefinitely. Supplementation with vitamin C, vitamin E, and selenium is recommended. Drugs and oxidants known to cause hemolysis and stressful catabolic states should be avoided. Oral administration of glutathione analogs has been tried with variable success.

Prenatal diagnosis can be achieved by the measurement of 5-oxoproline in amniotic fluid, by enzyme analysis in cultured amniocytes or chronic villus samples, or by *GSS* gene analysis. Successful pregnancy in an affected female (moderate form) has been reported, with favorable outcomes for both mother and infant.

Glutathione Synthetase Deficiency, Mild Form

The mild form has been reported in only a few patients. Mild to moderate hemolytic anemia has been the only clinical finding. Splenomegaly has been reported in some patients. Cognitive development is normal. Chronic metabolic acidosis typically is not seen. Some patients can have increased concentrations of 5-oxoproline in the urine. Biallelic pathogenic variants in *GSS*, the gene encoding the enzyme, appear to decrease the half-life of the enzyme, causing an increased rate of protein turnover without affecting its catalytic function. The expedited rate of enzyme turnover caused by these pathogenic variants is of little or no consequence for tissues with protein synthetic capability. However, inability of mature erythrocytes to synthesize protein results in glutathione deficiency in the erythrocytes. **Treatment** is that of hemolytic anemia and avoidance of drugs and oxidants that can trigger the hemolytic process.

All forms of glutathione synthetase deficiency are inherited as an autosomal recessive trait. **Diagnosis** can be confirmed by *GSS* gene analysis or enzyme activity in erythrocytes or skin fibroblasts.

5-Oxoprolinase Deficiency

More than 20 patients with 5-oxoprolinuria (4-10 g/day) caused by 5-oxoprolinase (see Fig. 105.11) deficiency *(OPLAH)* have been described. No specific clinical picture has yet emerged; completely asymptomatic affected individuals have also been identified. It is therefore not clear whether 5-oxoprolinase deficiency is of any clinical consequence. No treatment is currently recommended.

γ-Glutamylcysteine Synthetase Deficiency (Glutamate-Cysteine Ligase Deficiency)

γ-Glutamylcysteine synthetase deficiency is an autosomal recessive disorder caused by biallelic pathogenic variants in *GCLC*. Only a few patients with this enzyme deficiency have been reported. The most consistent clinical manifestation has been mild chronic hemolytic anemia. Acute attacks of hemolysis have occurred after exposure to sulfonamides. Peripheral neuropathy and progressive spinocerebellar degeneration have been noted in two siblings in adulthood. Laboratory findings of chronic hemolytic anemia were present in all patients. Generalized aminoaciduria is also found because the γ-glutamyl cycle is involved in amino acid transport in cells (see Fig. 105.11). **Treatment** focuses on the management of hemolytic anemia and avoidance of drugs and oxidants that may trigger the hemolytic process.

γ-GLUTAMYL TRANSPEPTIDASE DEFICIENCY (GLUTATHIONEMIA)

γ-Glutamyl transpeptidase (GGT) is expressed in any cell that has secretory or absorptive functions. It is especially abundant in the kidneys, pancreas, intestines, and liver. The enzyme is also present in the bile. Measurement of GGT in the blood is frequently performed to evaluate for liver and bile duct diseases.

GGT deficiency causes elevation in glutathione concentrations in body fluids, but the cellular levels remain normal (see Fig. 105.11). Because only a few patients with GGT deficiency have been reported, the scope of clinical manifestations has not yet been defined. Mild to moderate intellectual disability and severe behavioral problems were observed in three patients. However, one of two sisters with this condition had normal intelligence as an adult, and the other had Prader-Willi syndrome.

Laboratory findings include marked elevations in urinary glutathione (up to 1 g/day), γ-glutamylcysteine, and cysteine. None of the reported patients have had generalized aminoaciduria, a finding that would have been expected to occur in this enzyme deficiency (see Fig. 105.11).

Diagnosis can be confirmed by measurement of the enzyme activity in leukocytes or cultured skin fibroblasts. *No effective treatment has been proposed.* The condition is inherited as an apparent autosomal recessive trait. The γ-glutamyl transpeptidases represent a large family of enzymes encoded by at least seven genes.

GENETIC DISORDERS OF METABOLISM OF γ-AMINOBUTYRIC ACID

See Chapter 105.11.

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105.11 Disorders of Neurotransmitter Metabolism

Oleg A. Shchelochkov and Charles P. Venditti

Neurotransmitters are chemical substances released from the axonal end of excited neurons at the synaptic junctions; they mediate initiation, amplification, or inhibition of neural impulses. Several amino acids and their metabolites act as neurotransmitters in the central and peripheral nervous system. Pathogenic variants in genes responsible for the synthesis, transport, or degradation of these substances may cause conditions that manifest neurologic and/or psychiatric abnormalities (Table 105.3). Previously, children affected by disorders of neurotransmitters have been given syndromic diagnoses such as cerebral palsy, epilepsy, parkinsonism, dystonia, or autism. Diagnosis, in most cases, requires specialized laboratory studies of the CSF, because some of the neurotransmitters generated in the CNS, dopamine and serotonin, do not cross the BBB, and their abnormal concentrations are not detected in the serum or urine.

TYROSINE HYDROXYLASE DEFICIENCY (INFANTILE PARKINSONISM, AUTOSOMAL RECESSIVE DOPA-RESPONSIVE DYSTONIA, AUTOSOMAL RECESSIVE SEGAWA SYNDROME)

Tyrosine hydroxylase catalyzes the formation of L-dopa from tyrosine. Deficiency of this enzyme results in deficiencies of dopamine and norepinephrine (see Fig. 105.2 and Fig. 105.12). The differential diagnosis includes a wide range of inherited dystonias, including autosomal dominant dystonia caused by GTP cyclohydrolase 1 deficiency.

Clinical manifestations range from mild to very severe. In general, two phenotypes have been recognized. In the **mild** form (dopa-responsive dystonia, or **type A**), symptoms of unilateral limb dystonia causing gait incoordination and postural tremor occur in childhood and worsen with age when the condition remains untreated. Diurnal variation of symptoms (worse at the end of the day) may be present. Cognitive development is usually normal.

In the **severe** form of tyrosine hydroxylase deficiency (infantile parkinsonism, infantile encephalopathy, or **type B**), the clinical manifestations occur at birth or shortly thereafter and include microcephaly, developmental delay, involuntary movements of the limbs with spasticity, dystonia, ptosis, expressionless face, oculogyric crises (upward eye-rolling movements), and autonomic dysfunction (temperature instability, excessive sweating, hypoglycemia, salivation, tremor, gastrointestinal reflux, constipation). Brisk reflexes, myoclonus, athetosis, and distal chorea may be present. The patient with the severe form usually shows incomplete response to treatment with L-dopa and is prone to developing L-dopa–induced dyskinesia as a side effect.

Laboratory findings include reduced levels of dopamine and its metabolite homovanillic acid (HVA) and normal concentrations of tetrahydrobiopterin (BH_4), neopterin, and 5-hydroxyindoleacetic acid (5-HIAA, a metabolite of serotonin) in the CSF. Serum prolactin levels are usually elevated. These findings are not diagnostic of the condition; diagnosis should be established by molecular gene analysis.

Treatment with L-dopa/carbidopa results in significant clinical improvement in most patients, but the severe forms are invariably associated with L-dopa–induced dyskinesias. To minimize the side effects of therapy, treatment should be started with a low dose and, if needed, increased very slowly. Other therapeutic interventions include anticholinergics, serotonergic agents, and monoamine oxidase (MAO) B inhibitors, including amantadine, biperiden, and selegiline. Bilateral subthalamic nucleus deep brain stimulation has shown clinical efficacy in one case. Tyrosine hydroxylase deficiency is inherited as an autosomal recessive trait. Molecular testing for pathogenic variants in the *TH* gene is available clinically.

AROMATIC L-AMINO ACID DECARBOXYLASE DEFICIENCY

Aromatic L-amino acid decarboxylase (AADC encoded by *DDC*) is a vitamin B_6–dependent enzyme that catalyzes the decarboxylation of both 5-hydroxytryptophan to form serotonin (see Fig. 105.5 and Fig. 105.12) and L-dopa to generate dopamine (see Fig. 105.2 and Fig. 105.12). **Clinical manifestations** of this autosomal recessive disorder reflect the reduced availability of dopamine and serotonin. Poor feeding, lethargy, hypotension, hypothermia, oculogyric crises, and ptosis have been observed in affected neonates. Clinical findings in infants and older children include developmental delay, truncal hypotonia with hypertonia of limbs, oculogyric crises, extrapyramidal movements (choreoathetosis, dystonia, myoclonus), and autonomic abnormalities (sweating, salivation, irritability, temperature instability, hypotension). Symptoms may have a diurnal variation, becoming worse by the end of the day.

Laboratory findings include decreased concentrations of dopamine and serotonin and their metabolites (HVA, 5-HIAA, norepinephrine, vanillylmandelic acid [VMA]) and increased levels of 5-hydroxytryptophan, L-dopa, and its metabolite (3-*O*-methyldopa) in body fluids, especially in CSF. Elevated serum concentrations of prolactin (the result of dopamine deficiency) have also been observed. Brain MRI reveals cerebral atrophy with degenerative changes in the white matter. A urine screening program, focused on 3-*O*-methyl-dopa and VMA, has demonstrated diagnostic promise in high-disease-prevalence populations.

Treatment with neurotransmitter precursors has produced limited clinical improvement. Dopamine and serotonin have no therapeutic value because of their inability to cross the BBB. Nonergot dopamine agonists, MAO inhibitors (tranylcypromine), serotonergic agents, and high doses of pyridoxine/pyridoxal phosphate, a cofactor for the AADC enzyme, are preferred. The demonstration of putamen-directed gene therapy with an adeno-associated viral vector has shown some benefit in patients. Preimplantation genetic diagnosis after in vitro fertilization has been achieved in the high-prevalence Taiwanese population.

TETRAHYDROBIOPTERIN DEFICIENCY

See Chapter 105.1.

Tetrahydrobiopterin (BH_4) is the enzymatic cofactor for phenylalanine hydroxylase (see Fig. 105.1 and Fig. 105.12), tyrosine hydroxylase

Table 105.3 Neurotransmitter Disorders Affecting Biogenic Amines and GABA Metabolism and Transport: Biomarker Assessment in Biologic Fluids

CSF MARKER	DISEASE OMIM[A]/GENE	BIOCHEMICAL PATTERN
Biogenic amines	TH deficiency 191290/*TH*	*CSF*:↓ HVA, MHPG, and HVA/5-HIAA ratio
	AADC deficiency 107930/*DDC*	*CSF*: ↓↓ HVA and 5-HIAA; ↑↑ 3OMD and 5HTP *Urine*: ↑ vanillactate; *blood*: ↑ 3OMD
	MAOA deficiency 309850/*MAO-A*	*CSF*: ↓↓ 5-HIAA and HVA *Plasma/urine*: ↑ catecholamines and serotonin
	DBH deficiency 609312/*DBH*	*CSF*: ↑ HVA, HVA/5-HIAA ratio, ↓ MHPG[a] *Urine/plasma*: ↓↓ norepinephrine and epinephrine; ↑ dopamine and DOPAC
	DAT1 deficiency 126455/*SLC6A3*	*CSF*: ↑ HVA and HVA/5-HIAA ratio
	VMAT2 deficiency 193001/SLC18A2	*Urine*: ↑ HVA and 5-HIAA ↓ Norepinephrine and dopamine
Pterins	Dominant GTPCH-I deficiency 600225/*GCH*	*CSF*: ↓ NP, BP, BH_4, HVA, and 5-HIAA
	SR deficiency 182125/*SRD*	*CSF*: ↑ BP and SP; normal NP; ↓ BH_4, HVA, and 5-HIAA *Urine*: ↑ SP
GABA	SSADH deficiency 610045/*ALDH5A1*	*CSF*: ↑ GABA *Plasma and urine*: ↑↑ GHB

[a]Expected values.

↑: Increased values compared with reference aged values; ↓: decreased values compared with reference aged values.

5-HIAA, 5-Hydroxyindoleacetic acid; AADC, aromatic L-amino acid decarboxylase; BP, biopterin; CSF, cerebrospinal fluid; DBH, dopamine β-hydroxylase; DOPAC, dihydroxyphenylacetic acid; GHB, γ-hydroxybutyrate; GTPCH-I, guanosine triphosphate cyclohydrolase-I; HVA, homovanillic acid; MAOA, monoamine oxidase A; MHPG, 3-methoxy-4-hydroxyphenylethyleneglycol; NP, neopterin; SP, sepiapterin; SR, sepiapterin reductase; SSADH, succinic semialdehyde dehydrogenase; TH, tyrosine hydroxylase.

From García-Cazorla À, Artuch R. Neurotransmitter disorders. In Rosenberg RN, Pascual JM, eds. *Rosenberg's Molecular and Genetic Basis of Neurological and Psychiatric Disease*, 6th ed. Vol. 1. London: Elsevier; 2020: Table. 67.1.

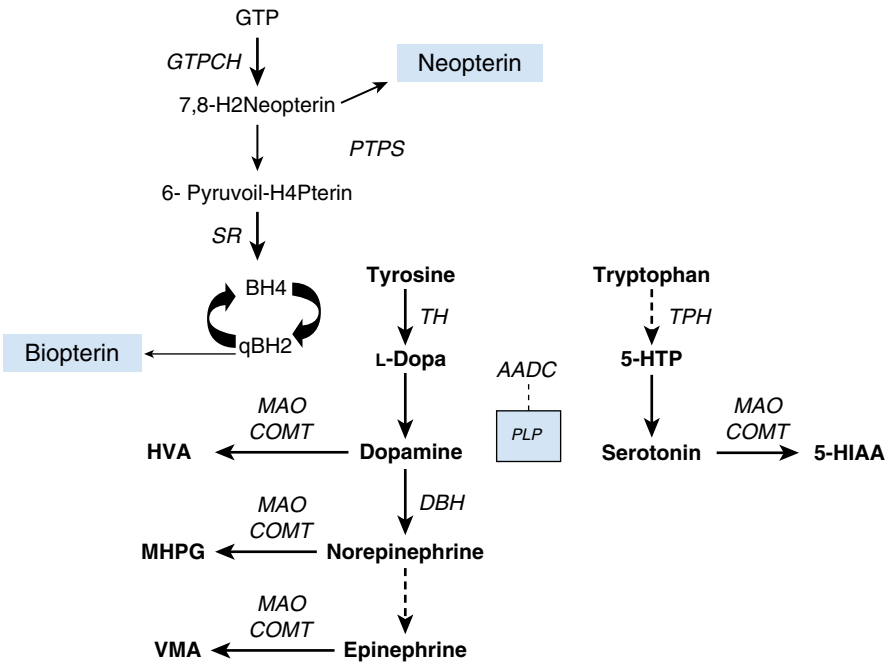

Fig. 105.12 Metabolic pathways of monoamines. The first step in their formation is catalyzed by amino acid–specific hydroxylases, which require tetrahydrobiopterin (BH_4) as a cofactor. The synthesis of BH_4 comes from GTP, and it is initiated by the enzyme GTPCH-I, which forms dihydroneopterin triphosphate. Levodopa and 5-HTP are metabolized by a common B_6-dependent AADC into dopamine and serotonin. MAOA catabolizes adrenaline and noradrenaline to VMA and MHPG. This enzyme is also involved in the catabolism of both dopamine into HVA and serotonin into 5-HIAA. 5-HIAA, 5-Hydroxyindoleacetic acid; 5-HTP, 5-hydroxytryptophan; AADC, aromatic l-amino acid decarboxylase; GTP, guanosine triphosphate; GTPCH-I, GTP cyclohydrolase-1; HVA, homovanillic acid; MAOA, monoamine oxidase A; MHPG, 3-methoxy-4-hydroxyphenylethyleneglycol; VMA, vanillylmandelic acid. (*From García-Cazorla À, Artuch R. Neurotransmitter disorders. In: Rosenberg RN, Pascual JM, eds. Rosenberg's Molecular and Genetic Basis of Neurological and Psychiatric Disease, 6th ed. Vol. 1. London: Elsevier; 2020: Fig. 67.1.*)

(see Fig. 105.2 and 105.12), tryptophan hydroxylase (see Fig. 105.5 and 105.12), and nitric oxide synthase. It is synthesized from GTP in many tissues (see Fig. 105.1). Deficiencies of enzymes involved in the biosynthesis of BH_4 result in inadequate production of this cofactor, which causes deficiencies of monoamine neurotransmitters with or without concomitant hyperphenylalaninemia.

Tetrahydrobiopterin Deficiency with Hyperphenylalaninemia

See Chapter 105.1.

Tetrahydrobiopterin Deficiency Without Hyperphenylalaninemia

GTP Cyclohydrolase 1 Deficiency (Hereditary Progressive Dystonia, Autosomal Dominant Dopa-Responsive Dystonia, Autosomal Dominant Segawa Syndrome)

Guanosine triphosphate (GTP) cyclohydrolase 1 catalyzes the first and rate-limiting step in the biopterin biosynthesis pathway (see Fig. 105.1). This form of dystonia, caused by GTP cyclohydrolase 1 deficiency *(GCH1)*, has an autosomal dominant mode of inheritance and is more common in females than in males (4:1 ratio) (see Chapter 637.4). **Clinical manifestations** usually start in early childhood with tremor and dystonia of the lower limbs **(toe gait)**, which may spread to all extremities within a few years. Torticollis, dystonia of the arms, and poor coordination may precede dystonia of the lower limbs. Early development is generally normal. Symptoms have an impressive diurnal variation, becoming worse by the end of the day and improving with sleep. Autonomic instability is common. Parkinsonism may also be present or develop with advancing age. Late presentation in adult life has also been reported, associated with **action dystonia** ("writer's cramp"), torticollis, or generalized rigid hypertonia with tremor but without postural dystonia. Additionally, limited data on adults suggest symptoms related to serotonin deficiency (sleep disturbance, cognitive impairment, impulsivity).

Laboratory findings show reduced levels of BH_4 and neopterin in the CSF without hyperphenylalaninemia (not to be confused with the autosomal recessive form of BH_4-deficient hyperphenylalaninemia, see Chapter 105.1). Dopamine and its metabolite (HVA) may also be reduced in CSF. The serotonergic pathway is less affected by this enzyme deficiency; thus concentrations of serotonin and its metabolites are usually normal. Plasma phenylalanine is normal, but an oral phenylalanine loading test (100 mg/kg) produces an abnormally high plasma phenylalanine level with an elevated phenylalanine/tyrosine ratio. The ratio, obtained 2-3 hours after the load, in combination with urine neopterin level, has optimal diagnostic specificity and sensitivity. The existence of asymptomatic carriers indicates that other factors or genes may play a role in pathogenesis. Asymptomatic carriers may be identified using molecular testing or by the phenylalanine loading test. **Diagnosis** is confirmed by demonstrating reduced levels of BH_4 and neopterin in CSF, measurement of the enzyme activity, and molecular genetic analysis of *GCH1* (see Chapter 105.1). Clinically, the condition should be differentiated from other causes of dystonias and childhood parkinsonism, especially tyrosine hydroxylase, sepiapterin reductase, and aromatic amino acid decarboxylase deficiencies.

Treatment with L-dopa/carbidopa usually produces dramatic clinical improvement. Oral administration of BH_4 is also effective but is rarely used.

Sepiapterin Reductase Deficiency

Sepiapterin reductase (encoded by *SPR*) is involved in the conversion of 6-pyruvoyl-tetrahydropterin to BH_4. It also participates in the salvage pathway of BH_4 synthesis (see Fig. 105.1 and Fig. 105.12). Sepiapterin reductase deficiency, an autosomal recessive condition, results in accumulation of 6-lactoyl-tetrahydropterin, which can be converted to sepiapterin nonenzymatically. The majority of sepiapterin is metabolized to BH_4 through the salvage pathway in peripheral tissues (see Fig. 105.1 and Fig. 105.12), but because of the low activity of dihydrofolate reductase in the brain, the amount of BH_4 remains insufficient for proper synthesis of dopamine and serotonin. This explains the absence of hyperphenylalaninemia and the often-delayed diagnosis.

Clinical manifestations in severely affected patients usually appear within a few months of life. Cardinal manifestations include paroxysmal stiffening, oculogyric crises, and hypotonia. Additional findings include motor and language delays, weakness, limb hypertonia, dystonia, hyperreflexia, and early-onset parkinsonism. The symptoms usually have a diurnal variation. Misdiagnosis as cerebral palsy is common, and a wide variability of symptoms has been reported. **Diagnosis** is established by measurement of CSF neurotransmitters and pterin metabolites, which reveal decreased dopamine, HVA, norepinephrine, and 5-HIAA and marked elevations of sepiapterin and dihydrobiopterin. The serum concentration of prolactin may be elevated. The phenylalanine loading test may have diagnostic utility, but it is being replaced by molecular genetic analysis, which can confirm the diagnosis. **Treatment** with slowly increasing doses of L-dopa/carbidopa and 5-hydroxytryptophan usually produces dramatic clinical improvement.

DOPAMINE β-HYDROXYLASE DEFICIENCY

Dopamine β-hydroxylase catalyzes the conversion of dopamine to norepinephrine (see Fig. 105.2 and Fig. 105.12). The deficiency of this enzyme results in reduced or absent synthesis of norepinephrine, leading to dysregulation of the sympathetic function. Infants and children may present with difficulty opening the eyes, ptosis, hypotension, hypothermia, hypoglycemia, and nasal stuffiness. Adult patients may present with profound deficits of autonomic regulation, resulting in severe orthostatic hypotension, and sexual dysfunction in males. Presyncopal symptomatology includes dizziness, blurred vision, dyspnea, nuchal discomfort, and chest pain; olfactory function remains relatively intact. The **diagnosis** can be aided by performing autonomic function testing (measurement of the sinus arrhythmia ratio, blood pressure studies during controlled hyperventilation, Valsalva maneuver, cold pressor, handgrip exercise). **Laboratory findings** include decreased or absent norepinephrine and epinephrine and their metabolites, with elevated levels of dopamine and its metabolite (HVA), in plasma, CSF, and urine. Elevated plasma dopamine may be pathognomonic for this disease. MRI of the brain shows decreased brain volume, consistent with the neurotrophic role of norepinephrine. **Treatment** with L-dihydroxyphenylserine, which is converted to norepinephrine directly in vivo by the action of AADC, leads to significant improvement in orthostatic hypotension and normalizes noradrenaline and its metabolites. The condition is inherited as an autosomal recessive trait. Dopamine β-hydroxylase is encoded by *DBH*.

MONOAMINE OXIDASE A DEFICIENCY

The human genome encodes two MAO isoenzymes: MAO A and MAO B. Both enzymes catalyze oxidative deamination of most biogenic amines in the body, including serotonin (see Fig. 105.5 and Fig. 105.12), norepinephrine, epinephrine, and dopamine (see Fig. 105.2 and Fig. 105.12). The genes for both isoenzymes are on the X chromosome (Xp11.3). A deletion of both genes can also encompass a neighboring gene, *NDP,* resulting in a contiguous deletion syndrome, which can present as an atypical **Norrie disease** (see Chapter 640). Male patients with MAO A deficiency manifest borderline intellectual deficiency and impaired impulse control. The consequences of the isolated MAO B deficiency are incompletely understood. Combined MAO A and B deficiency causes severe intellectual disability and behavioral problems and can be associated with pronounced laboratory abnormalities (e.g., fourfold to sixfold serotonin elevation in physiologic fluids, elevated *O*-methylated amine metabolites, and reduced deamination products [VMA, HVA]). Dietary intervention (low tyramine, phenylethylamine, and L-dopa/dopamine intake) did not improve patients' blood serotonin levels. Inheritance of MAO deficiency is X-linked. **Treatment** of MAO A deficiency is supportive.

DISORDERS OF GABA METABOLISM

GABA is the main inhibitory neurotransmitter synthesized in the synapses through decarboxylation of glutamic acid by glutamate decarboxylase (GAD). The same pathway is responsible for production of GABA in other organs, especially the kidneys and the β cells of the pancreas. The GAD enzyme requires pyridoxine (vitamin B_6) as a cofactor.

Two GAD enzymes, GAD1 (GAD_{67}) and GAD2 (GAD_{65}), have been identified. **GAD1** is the main enzyme in the brain, and **GAD2** is the major enzyme in the β cells. Antibodies against GAD_{65} and GAD_{67} have been implicated in the development of type 1 diabetes and **stiff-person syndrome**, respectively. GABA is catabolized to succinic acid by two enzymes: GABA transaminase and succinic semialdehyde dehydrogenase (SSADH) (see Figs. 105.11 and 105.12).

GABA Transaminase Deficiency

Clinical manifestations in the two index infant siblings included severe developmental delay and intellectual disability, hypotonia, hyperreflexia, lethargy, refractory seizures, and increased linear growth likely related to GABA-mediated increased secretion of growth hormone. Increased concentrations of GABA and β-alanine were found in the CSF (see Fig. 105.11 and Fig. 105.12). Evidence of leukodystrophy was noted in the postmortem examination of the brain. A third patient showed severe psychomotor retardation, recurrent episodic lethargy, and intractable seizures with comparable CSF metabolite abnormalities to those of the index probands. GABA transaminase deficiency is demonstrated in the brain and lymphocytes. **Treatment** is symptomatic. Intervention with vitamin B_6, the cofactor for the enzyme, was without therapeutic benefit. The condition is inherited in the autosomal recessive manner and caused by biallelic pathogenic variants in *ABAT*.

Succinic Semialdehyde Dehydrogenase Deficiency (β-Hydroxybutyric Aciduria)

Clinical manifestations of SSADH deficiency, an autosomal recessive disorder, usually begin in infancy with developmental delays with a disproportionate deficit in expressive language, hypotonia, and ataxia; seizures occur in approximately 50% of patients (see Fig. 105.11 and Fig. 105.12). Many patients also carry the diagnosis of **autism spectrum disorder**. Neuropsychiatric comorbidity (especially oppositional defiance, obsession-compulsion, and hyperactivity) can be disabling, particularly in adolescents and adults. Abnormal EEG findings include background slowing and generalized spike-wave paroxysms, with variable lateralization in hemispheric onset and voltage predominance. Photosensitivity and electrographic status epilepticus of sleep have been reported in combination with difficulties in sleep maintenance and excessive daytime somnolence. Brain MRI shows an increased T2-weighted hyperintensity involving the globus pallidi, cerebellar dentate nuclei, and subthalamic nuclei, usually in a bilaterally symmetric distribution.

The biochemical hallmark, γ-hydroxybutyric acid (GHB), is elevated in physiologic fluids (CSF, plasma, urine) in all patients. Increased concentrations of GABA are also found in the CSF. Heightened diagnostic suspicion evolves through documentation of elevated urinary GHB, and confirmation is achieved by molecular genetic testing.

Effective **treatment** is lacking. Vigabatrin (a GABA-transaminase inhibitor) has been employed empirically, with mixed outcomes, and there is concern with its use, as it further elevates CNS GABA in an already hyper-GABAergic disorder. Additionally, vigabatrin can cause constriction of the visual field, and long-term use is contraindicated.

SSADH is encoded by *ALDH5A1,* and inheritance follows an autosomal recessive pattern. **Prenatal diagnosis** has been achieved by measurement of GHB in the amniotic fluid, assay of the enzyme activity in the amniocytes, chorionic villus sampling, or DNA analysis.

DEFECTS IN NEUROTRANSMITTER TRANSPORTER PROTEINS

More than 20 different proteins are involved in transporting neurotransmitters across the neuronal membranes. The main function of most of these transporters is to remove excess neurotransmitters from the synaptic junction into the presynaptic neurons (reuptake). This recycling process not only regulates the precise effect of neurotransmitters at the synaptic junction but also resupplies the presynaptic neurons with neurotransmitters for future use. A few transporter proteins are involved in shuttling neurotransmitters from the neuronal cytoplasm across the membrane of synaptic vesicles for storage (vesicular transporters). On neuronal stimulation, these vesicles release a bolus of neurotransmitters through exocytosis. As expected, pathogenic variants in transporter proteins interfere with the proper reuptake and storage of neurotransmitters and may result in clinical manifestations similar to those seen in deficiencies of neurotransmitter metabolism. Several conditions caused by pathogenic variants of neurotransmitter protein transporters have been described, including dopamine transporter protein deficiency and dopamine-serotonin vesicular transporter disease.

SLC6A3-Related Dopamine Transporter Protein Deficiency

This transporter protein is involved in the reuptake of dopamine by the presynaptic neurons, and its deficiency causes depletion of dopamine and thus a dopamine-deficient state. The dopamine transporter protein (DAT) is encoded by *SLC6A3*. Children with biallelic pathogenic variants in *SLC6A3* present with symptoms of infantile **parkinsonism-dystonia syndrome**. Irritability and feeding difficulties start shortly after birth and progress to hypotonia, lack of head control, parkinsonism, dystonia, and global developmental delay by early infancy. Brain MRI usually shows no abnormalities.

CSF examination reveals elevation of HVA and a normal level of 5-HIAAs. The urinary level of HVA and serum concentration of prolactin are increased. Diagnosis can be established by demonstrating loss-of-function pathogenic variants in the *SLC6A3* gene.

Dopamine-Serotonin Vesicular Transporter Disease (Vesicular Monoamine Transporter Deficiency)

This autosomal recessive condition is caused by pathogenic variants in the *SLC18A2* gene. This gene encodes the vesicular monoamine transporter 2 (VMAT2) involved in transporting dopamine and serotonin from the cytoplasm into the synaptic storage vesicles located in the axonal terminals of the presynaptic neurons. Most affected children presented in the first year of life with symptoms consistent with deficiencies of dopamine (hypotonia progressing into dystonia, parkinsonism, oculogyric crises), serotonin (sleep and psychiatric disturbances), and norepinephrine-epinephrine (excessive sweating, tremors, temperature instability, postural hypotension, ptosis). Neurocognitive delays become apparent in the first year of life. No diurnal variation of the symptoms was noted. Brain imaging studies were within normal limits. Changes in the levels of CNS neurotransmitters and their metabolites have been inconsistent.

The phenotype resembles that seen in AADC and BH_4 deficiencies. Diagnosis requires demonstration of biallelic pathogenic variants in *SLC18A2*. **Treatment** with L-dopa/carbidopa caused exacerbation of symptoms, whereas pramipexole, a dopamine receptor agonist, has resulted in a promising clinical response.

HISTIDINE DECARBOXYLASE DEFICIENCY

Decarboxylation of histidine by histidine decarboxylase produces histamine, which functions as a neurotransmitter in the brain. Deficiency of this enzyme (expressed mainly in the posterior hypothalamus) results in deficiency of histamine in the CNS and in one family has been reported as an autosomal dominant form of Tourette syndrome (see Chapter 105.13).

HYPERPROLINEMIA

Intellectual disability and seizures are common findings in most patients with hyperprolinemia types I and II. Patients with **type I** hyperprolinemia typically show a benign clinical course but could have an increased risk of developing schizophrenia. The contribution of increased concentrations of proline to the mechanisms of schizophrenia, however, remains unclear. The neurologic abnormalities observed in hyperprolinemia **type II** are mainly caused by development of vitamin B_6 dependency in this condition (see Chapter 105.9). Dietary intervention in hyperprolinemia type I and II is neither feasible nor recommended.

3-PHOSPHOGLYCERATE DEHYDROGENASE DEFICIENCY

See Chapter 105.8.

PHOSPHOSERINE AMINOTRANSFERASE DEFICIENCY

See Chapter 105.8.

NONKETOTIC HYPERGLYCINEMIA

See Chapter 105.7.

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105.12 Urea Cycle and Hyperammonemia (Arginine, Citrulline, Ornithine)

Oleg A. Shchelochkov and Charles P. Venditti

Catabolism of amino acids results in the production of free ammonia, which in high concentrations is toxic to the CNS. Mammals detoxify ammonia to urea through a series of reactions known as the **urea cycle** (Fig. 105.13). It is composed of five enzymes: carbamoyl phosphate synthetase 1 **(CPS1)**, ornithine transcarbamylase **(OTC)**, argininosuccinate synthetase **(ASS)**, argininosuccinate lyase **(ASL)**, and arginase 1 **(ARG1)**. A sixth enzyme, *N*-acetylglutamate (NAG) synthetase **(NAGS)**, catalyzes synthesis of NAG, an obligatory activator (effector) of the CPS1 enzyme. Individual deficiencies of these enzymes have been observed and, with an overall estimated prevalence of 1 in 35,000 live births, they are the most common genetic causes of hyperammonemia in infants (Table 105.4).

GENETIC CAUSES OF HYPERAMMONEMIA

Hyperammonemia, sometimes severe, occurs in inborn errors of metabolism other than the urea cycle defects (Table 105.5; see also Table 104.5). The mechanisms of hyperammonemia in some of these conditions are diverse and include accumulation of toxic metabolites (e.g., organic acids), impaired transport of urea cycle intermediates (e.g., HHH syndrome), or depletion of urea cycle intermediates (e.g., lysinuric protein intolerance), leading to compromised function of the urea cycle.

CLINICAL MANIFESTATIONS OF HYPERAMMONEMIA

In the **neonatal period**, symptoms and signs are mostly related to brain dysfunction and are similar regardless of the cause of the hyperammonemia. The affected infant appears healthy at birth but becomes symptomatic in the first days of life, often following the introduction of dietary protein. Refusal to eat, vomiting, tachypnea, and lethargy can quickly progress to a deep coma. Seizures are common. Physical examination may reveal hepatomegaly in addition to obtundation. Hyperammonemia can trigger increased intracranial pressure that may be manifested by a bulging fontanelle and dilated pupils.

In **infants and older children**, acute hyperammonemia is manifested by vomiting and neurologic abnormalities such as ataxia, confusion, agitation, irritability, combativeness, and psychosis. These manifestations may alternate with periods of lethargy and somnolence that may progress to coma.

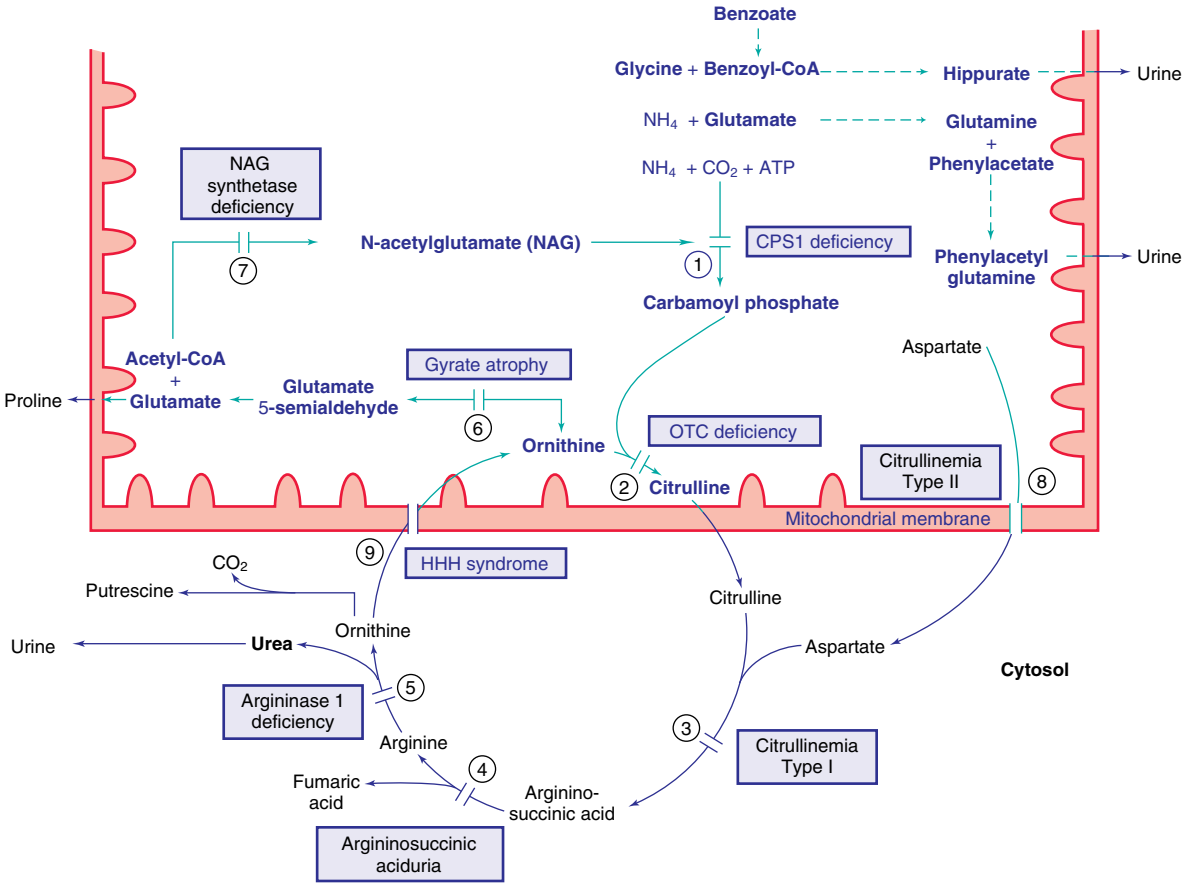

Fig. 105.13 Urea cycle: Pathways for ammonia disposal and ornithine metabolism. Reactions occurring in the mitochondria are depicted in *purple*. Reactions shown with *interrupted arrows* are the alternative pathways for the disposal of ammonia. **Enzymes:** *(1)* Carbamoyl phosphate synthetase type 1 (CPS1), *(2)* ornithine transcarbamylase (OTC), *(3)* argininosuccinate synthetase (ASS), *(4)* argininosuccinate lyase (ASL), *(5)* arginase 1, *(6)* ornithine aminotransferase, *(7)* *N*-acetylglutamate (NAG) synthetase, *(8)* citrin, *(9)* ornithine transporter (ORNT1). HHH syndrome, hyperammonemia-hyperornithinemia-homocitrullinemia.

Table 105.4 The Urea Cycle Disorders

ENZYME OR TRANSPORTER DEFICIENCY	GENE (INHERITANCE)	INCIDENCE	CLINICAL MANIFESTATIONS	AMINO ACIDS (PLASMA, UNLESS OTHERWISE INDICATED)	URINE ORGANIC ACIDS
N-Acetylglutamate synthetase MIM 237310	*NAGS* (AR)	<1:2,000,000	Acute episodic hyperammonemia Late-onset presentations	↓ Citrulline ↓ Arginine ↑ Glutamine	Unremarkable
Carbamyl phosphate synthetase I MIM 237300	*CPS1* (AR)	1:1,300,000	Acute episodic hyperammonemia Late-onset presentations	↓ Citrulline ↓ Arginine ↑ Glutamine	Unremarkable
Ornithine transcarbamylase MIM 311250	*OTC* (XL)	1:56,500	Acute episodic hyperammonemia Late-onset presentations	↓ Citrulline ↓ Arginine ↑ Glutamine	↑↑ Orotic acid
Argininosuccinate synthetase MIM 215700	*ASS1* (AR)	1:250,000	Acute episodic hyperammonemia Late-onset presentations	↑↑ Citrulline ↑ Glutamine ↓ Arginine	*N*-to-↑ orotic acid
Argininosuccinate lyase MIM 207900	*ASL* (AR)	1:218,750	Acute episodic hyperammonemia Late-onset presentations ↑ Neurodevelopmental issues Chronic liver disease Trichorrhexis nodosa	↑ Argininosuccinate and anhydrides ↑ Glutamine ↑ Citrulline ↓ Arginine	*N*-to-↑ orotic acid
Arginase MIM 207800	*ARG1* (AR)	1:950,000	Progressive spasticity (LL > UL) Usually mild ID Hyperammonemia (rare)	↑ Arginine	*N*-to-↑ orotic acid
Citrin MIM 605814, 603471	*SLC25A13* (AR)	<1:2,000,000	*NICCD* Intrahepatic cholestasis Poor growth Spontaneous improvement by 1 yr of age *FTTDCD* Failure to thrive Dyslipidemia Chronic liver disease *Citrullinemia type II* Acute episodic hyperammonemia Neuropsychologic manifestations	↑ Citrulline ↑ Arginine ↑ Methionine ↑ Threonine	Unremarkable
Ornithine transporter MIM 238970	*SLC25A15* (AR)	<1:2,000,000	Acute episodic hyperammonemia Liver failure (with or without hyperammonemia) Recurrent vomiting Neurologic presentation (e.g., DD, spasticity) Recurrent	↑ Ornithine ↑ Glutamine ↔-to-↓ citrulline ↑ Urine homocitrulline	*N*-to-↑ orotic acid

AR, Autosomal recessive; DD, developmental delay; FTTDCD, failure to thrive and dyslipidemia caused by citrin deficiency; IV, intravenous; LL, lower limb; NICCD, neonatal intrahepatic cholestasis caused by citrin deficiency; UL, upper limb; XL, X-linked.
From Rossignol F, Ah Mew N, Meltzer MR, Gropman AL. Urea cycle disorders. In: Rosenberg RN, Pascual JM, eds. *Rosenberg's Molecular and Genetic Basis of Neurological and Psychiatric Disease,* 6th ed. Vol. 1. London: Elsevier; 2020: Table 61.1.

Routine laboratory studies show no specific findings when hyperammonemia is caused by defects of the urea cycle enzymes. Blood urea nitrogen is usually low-normal or low in these patients. Some patients may initially present with unexplained elevated serum alanine transaminase (ALT) and aspartate transaminase (AST) and even meet the criteria for acute liver failure. In infants with organic acidemias, hyperammonemia is commonly associated with severe *acidosis* and *ketonuria*. Newborn infants with hyperammonemia are often misdiagnosed as having sepsis; they may succumb without a correct diagnosis. Neuroimaging may reveal cerebral edema. Autopsy may reveal microvesicular steatosis, mild cholestasis, and fibrosis of the liver. Thus because of the nonspecific presentation or urea cycle disorders, it is imperative to measure plasma ammonia levels in any ill infant with severe sepsis, unexplained liver dysfunction, recurrent emesis, or progressive encephalopathy.

DIAGNOSIS

The main criterion for diagnosis is hyperammonemia. Each clinical laboratory should establish its own normal values for blood ammonia. Normal newborn values are higher than those of the older child or adult. Occasionally, levels as high as 100 μmol/L can occur in healthy term infants. An ill infant usually manifests a blood ammonia level >150 μmol/L. Figure 105.14 illustrates an approach to the differential diagnosis of hyperammonemia in the newborn infant. Careful inspection of individual plasma amino acid profiles usually reveals abnormalities that assist in diagnosis. In patients with deficiencies of CPS1, OTC, or NAGS, frequent findings include elevations in plasma glutamine and alanine with concurrent decrements in citrulline and arginine. These disorders cannot be differentiated from one another by plasma amino acid levels alone. A marked increase in urinary orotic acid in patients with OTC deficiency helps differentiate this defect from CPS1 deficiency (see Table 105.4). Differentiation between the CPS1 deficiency and the NAGS deficiency may require molecular analysis of the relevant genes or, infrequently, an assay of the respective enzyme. Significant clinical improvement occurring after oral administration of carbamylglutamate, however, supports the diagnosis of NAGS deficiency. Patients with a deficiency of ASS, ASL, or arginase 1 have marked increases in the plasma levels of citrulline, argininosuccinic

Table 105.5 Inborn Errors of Metabolism Causing Hyperammonemia

DEFICIENCIES OF THE UREA CYCLIC ENZYMES
Carbamyl phosphate synthetase 1
Ornithine transcarbamylase
Argininosuccinate synthetase
Argininosuccinate lyase
Arginase 1
N-acetylglutamate synthetase
ORGANIC ACIDEMIA
Propionic acidemia
Methylmalonic acidemia
Isovaleric acidemia
β-Ketothiolase deficiency
Multiple carboxylase deficiencies
Medium-chain fatty acid acyl-CoA dehydrogenase deficiency
Glutaric acidemia type I
3-Hydroxy-3-methylglutaric aciduria
OTHERS
Lysinuric protein intolerance
Hyperammonemia-hyperornithinemia-homocitrullinemia syndrome
Transient hyperammonemia of the newborn
Congenital hyperinsulinism with hyperammonemia
Carbonic anhydrase VA deficiency

Table 105.6 Treatment of Acute Hyperammonemia in an Infant

1. Provide adequate calories, fluid, and electrolytes intravenously (10% glucose, NaCl* and intravenous lipids 1 g/kg/day). Add minimal amounts of protein, preferably as a mixture of essential amino acids (0.25 g/kg/day) during the first 24 hr of therapy.
2. Give priming doses of the following compounds (to be added to 20 mL/kg of 10% glucose and infused within 1-2 hr):
   - Sodium benzoate 250 mg/kg†
   - Sodium phenylacetate 250 mg/kg†
   - Arginine hydrochloride 200-600 mg/kg as a 10% solution
3. Continue infusion of sodium benzoate† (250-500 mg/kg/day), sodium phenylacetate† (250-500 mg/kg/day), and arginine (200-600 mg/kg/day‡) following the above priming doses. These compounds should be added to the daily intravenous fluid.
4. Initiate peritoneal dialysis or hemodialysis if above treatment fails to produce an appreciable decrease in plasma ammonia.

*The concentration of sodium chloride should be calculated to be 0.45–0.9%, including the amount of the sodium in the drugs.
†Sodium from these drugs should be included as part of the daily sodium requirement.
‡The higher dose of the range is recommended in the treatment of patients with citrullinemia and argininosuccinic aciduria. Arginine is not recommended in patients with arginase deficiency and in those whose hyperammonemia is secondary to organic acidemia. Sodium benzoate and sodium phenylacetate should be used with caution in patients with organic acidemias.

acid, or arginine, respectively. The combination of hyperammonemia and marked hypercitrullinemia or argininosuccinic acidemia is virtually pathognomonic for these disorders. Additional clinical clues may come from the past medical history. Children with urea cycle defects often self-select a low-protein, high-carbohydrate diet, especially those with late-onset disease or symptomatic females with partial OTC deficiency.

Because of the nonspecific clinical and laboratory findings, mass screening of newborn infants has been implemented in many countries, which can identify patients with ASS, ASL, and arginase 1 deficiencies.

TREATMENT OF ACUTE HYPERAMMONEMIA

The clinical outcome depends mainly on the severity and the duration of hyperammonemia. Serious neurologic sequelae are likely in newborns with severe elevations in blood ammonia (>300 μmol/L) lasting more than 12 hours. Thus acute hyperammonemia should be treated promptly and vigorously. The goal of therapy is to lower the concentration of ammonia. This is accomplished by (1) temporarily restricting dietary sources of ammonia (protein), (2) minimizing endogenous protein breakdown and favoring endogenous protein synthesis by providing adequate calories and essential amino acids, and (3) removal of ammonia from the body in a form other than urea (Table 105.6). Fluid, electrolytes, glucose (10–15%), and lipids (1-2 g/kg/day) should be infused intravenously, together with minimal amounts of protein (0.25 g/kg/day), preferably including essential amino acids. Oral feeding with a low-protein formula (0.5-1.0 g/kg/day) through a nasogastric tube should be started as soon as sufficient improvement is seen.

Because the kidneys clear ammonia poorly, its removal from the body must be expedited by formation of compounds with a high renal clearance. An important advance in the treatment of hyperammonemia has been the introduction of **nitrogen scavenging therapy** by using an exogenous organic acid that conjugates to endogenous nonessential amino acids (glycine and glutamine) to form nontoxic compounds with high renal clearance. The main organic acids used for this purpose are sodium salts of benzoic acid and phenylacetic acid. **Benzoate** forms hippurate through enzymatic conjugation with endogenous glycine in the liver (see Fig. 105.13). Each mole of benzoate removes one mole of ammonia as glycine. **Phenylacetate** enzymatically conjugates with glutamine to form phenylacetylglutamine readily excreted in the urine. One mole of phenylacetate can remove two moles of ammonia as glutamine from the body (see Fig. 105.13). Sodium phenylbutyrate, metabolized to phenylacetate, is the primary oral formulation. For intravenous (IV) use, a combined formulation of benzoate and phenylacetate (Ammonul) is commercially available.

Another valuable therapeutic adjunct is IV infusion of **arginine**, which is effective in all patients except those with arginase deficiency. Arginine administration supplies the urea cycle with ornithine (see Fig. 105.13). In patients with citrullinemia, one mole of arginine reacts with one mole of ammonia as carbamoyl phosphate to form citrulline. In patients with argininosuccinic acidemia, two moles of ammonia (as carbamoyl phosphate and aspartate) react with arginine to form argininosuccinic acid. Citrulline and arginosuccinate are less toxic than ammonia and more readily excreted by the kidneys. In patients with CPS1 or OTC deficiencies, arginine administration is indicated because this amino acid is not produced in sufficient amounts to enable endogenous protein synthesis. For enteral therapy, patients with OTC deficiency benefit from supplementation with *citrulline* (200 mg/kg/day) because one mole of citrulline reacts with one mole of ammonia (through aspartic acid) to form arginine. Administration of arginine or citrulline is contraindicated in patients with **arginase deficiency**, a rare condition in which the spastic diplegia is a much more common presenting feature rather than hyperammonemia. Arginine therapy is of no benefit if hyperammonemia is secondary to an organic acidemia. In a newborn infant with an initial episode of hyperammonemia, arginine should be used until the diagnosis is established (see Table 105.6).

Benzoate, phenylacetate, and arginine may be administered together for maximal therapeutic effect. A priming dose of these compounds is followed by continuous infusion until recovery from the acute state occurs. Both benzoate and phenylacetate are usually supplied as concentrated solutions and should be properly diluted (1–2% solution) for IV use. The recommended therapeutic doses of both compounds deliver a substantial amount of sodium to the patient; this amount should be included in the calculation of the daily sodium requirement. Benzoate and phenylacetate (or the combined formulation) should be used with caution in newborn infants with hyperbilirubinemia because they may displace bilirubin from albumin; however, there are no documented cases of kernicterus (see Chapter 123.4) reported in neonates with hyperammonemia who have received such therapies. In infants at risk, it is advisable to monitor and manage bilirubin levels while considering IV administration of benzoate or phenylacetate.

If the initial ammonia level is <500 μmol/L and if the foregoing therapies fail within 4-6 hours to produce any appreciable change in the blood ammonia level, **hemodialysis** should be considered. For patients

presenting with an ammonia level >500 μmol/L, extracorporeal detoxification is the initial method of ammonia removal. Exchange transfusion has little effect on reducing total body ammonia. It should be used only if dialysis cannot be employed promptly or when the patient is a newborn infant with significant hyperbilirubinemia (see earlier). Hemodialysis dramatically lowers blood ammonia within a few hours, but if it is unavailable or technically unfeasible, peritoneal dialysis may be used as a temporary solution. When hyperammonemia is caused by an organic acidemia and hemodialysis is not available, peritoneal dialysis can be used to remove both the offending organic acid and ammonia.

Oral administration of **neomycin** limits the growth of intestinal bacteria that can produce ammonia. However, this modality is of limited use in urea cycle patients (e.g., affected neonates) in whom reduction of hyperammonemia is an urgent priority. Oral **lactulose** acidifies the intestinal lumen, thereby reducing the diffusion of ammonia across the intestinal epithelium. This agent is of limited applicability in newborns, who have a high risk of acidemia and dehydration.

There may be considerable lag between the normalization of ammonia level and an improvement in the patient's neurologic status. Several days may be needed before the infant becomes fully alert.

There has been interest in the use of **cooling** as a therapeutic adjunct in newborn infants with metabolic encephalopathy such as that caused by hyperammonemia. Clinical studies are in progress to evaluate the efficacy of this approach.

Long-Term Therapy

Once the acute hyperammonemic episode is under control, maintenance therapy should be tailored to address the underlying cause of the hyperammonemia. All patients, regardless of the enzymatic defect, require protein restriction limited to the age-adjusted recommended dietary allowance (RDA). In pediatric patients with urea cycle defects, chronic administration of sodium benzoate (250 mg/kg/day), sodium phenylbutyrate (250-500 mg/kg/day), and arginine (200-400 mg/kg/day) or citrulline (in patients with OTC deficiency, 200-400 mg/kg/day) is effective in maintaining blood ammonia levels within the normal range (doses above are for patients who weigh <20 kg). Arginine and citrulline are contraindicated in patients with argininemia. Patients who have difficulty taking sodium phenylbutyrate can be trialed on glycerol phenylbutyrate. This compound conceals the offensive odor of sodium phenylbutyrate and may help with patient adherence. Benzoate and phenylacetate may lower carnitine levels, but clinical signs of carnitine deficiency or benefit from carnitine supplementation have not yet been demonstrated. These compounds have been used during pregnancy without obvious teratogenic effect. However, experience is still limited, and appropriate caution should be exercised.

Growth parameters, especially head circumference, and nutritional indices (blood albumin, prealbumin, pH, electrolytes, amino acids, zinc, selenium) should be followed closely. Long-term care of these patients is best achieved by a team of experienced professionals (pediatrician, nutritionist, child neurologist, metabolic geneticist). Skin lesions resembling **acrodermatitis enteropathica** (see Chapter 691) have been noted in a few patients with different types of urea cycle defects, presumably resulting from the deficiency of essential amino acids, caused by overzealous dietary protein restriction. Catabolic states (infections, fasting) that may trigger hyperammonemia should be avoided. They must be treated vigorously when they occur. It is important that all children with urea cycle defects avoid valproic acid because this drug can elevate blood ammonia even in some healthy individuals. In patients with CPS1, OTC, or ASS deficiency, acute hyperammonemic attacks may be precipitated by valproate administration.

CARBAMOYL PHOSPHATE SYNTHETASE 1 AND *N*-ACETYLGLUTAMATE SYNTHASE DEFICIENCIES

Without treatment, deficiencies of these two enzymes produce similar clinical and biochemical manifestations (see Figs. 105.13 and 105.14). There is a wide variation in the severity of symptoms and in the age at presentation. In near-complete enzymatic deficiency, symptoms appear during the first few days or even hours of life with signs and symptoms of hyperammonemia (refusal to eat, vomiting, lethargy, convulsion, coma). Increased intracranial pressure is frequent. Late forms (as late as the fourth decade of life) may present as an acute bout of hyperammonemia (lethargy, headache, seizures, psychosis) in a seemingly healthy individual. Coma and death may occur during these episodes. Diagnostic confusion with migraine is common. Intermediate forms with intellectual disability and chronic subclinical hyperammonemia interspersed with bouts of acute hyperammonemia have also been observed.

Laboratory findings include hyperammonemia. The plasma amino acid analysis typically shows a marked increase of glutamine and alanine with relatively low levels of citrulline and arginine. These are nondiagnostic changes that occur in hyperammonemia of diverse causes. Urinary orotic acid is usually low or may be absent (see Fig. 105.14).

Treatment of acute hyperammonemic attacks and the long-term therapy of the condition are outlined earlier (see Table 105.6). Treatment with oral carbamylglutamate of patients with NAGS deficiency can produce successful rescue of biochemical findings and symptoms. It is therefore important to differentiate between CPS1 and NAGS deficiencies by gene analysis of *CPS1* and *NAGS,* respectively.

CPS1 and NAGS deficiencies are both autosomal recessive conditions; the CPS1 enzyme is normally present in the liver and intestine. Neither of these conditions can be reliably identified by the mass screening of the newborn infants using current approaches.

ORNITHINE TRANSCARBAMYLASE DEFICIENCY

In this X-linked disorder, the hemizygous males are more severely affected than heterozygous females (see Figs. 105.13 and 105.14). The heterozygous females may have a mild form of the disease, but the majority (approximately 75%) remain asymptomatic, although investigations indicate subtle neurologic defects even in females without a discernable history of hyperammonemia. OTC deficiency is the most common form of all the urea cycle disorders, comprising approximately 40% of cases.

Clinical manifestations in a male newborn are usually those of **severe** hyperammonemia (see earlier) occurring in the first few days of life. **Mild forms**, such as in some heterozygous females, characteristically have episodic manifestations, which may occur at any age (usually after infancy). Episodes of hyperammonemia, manifested by vomiting and neurologic abnormalities (e.g., ataxia, mental confusion, agitation, combativeness, frank psychosis), are separated by periods of wellness. These episodes usually occur after ingestion of a high-protein diet or as a result of a catabolic state such as infection. Hyperammonemic coma, cerebral edema, and death may occur during one of these attacks. Cognitive development may proceed normally. Mild to moderate intellectual disability, however, is common. Gallstones have been seen in the survivors, although the mechanism remains unclear.

The major **laboratory finding** during the acute attack is hyperammonemia accompanied by marked elevations of plasma concentrations of glutamine and alanine with low levels of citrulline and arginine. The blood level of urea is usually low. A marked increase in the urinary excretion of orotic acid differentiates this condition from CPS1 deficiency (see Fig. 105.14). Orotate may precipitate in urine as pink-colored crystals. In the **mild form**, these laboratory abnormalities may revert to normal between attacks. This form should be differentiated from all the episodic conditions of childhood. Patients with lysinuric protein intolerance (see Chapter 105.14) may demonstrate some features of OTC deficiency, but the former can be differentiated by increased urinary excretion of lysine, ornithine, and arginine and elevated blood concentrations of citrulline.

The prevalence of OTC deficiency is 1 in 56,000 to 1 in 77,000 live births. This condition is not identifiable by the mass screening of newborn infants using current approaches. The **diagnosis** is most conveniently confirmed by *OTC* gene analysis. Many *OTC* pathogenic variants (>300) have been identified. Genotype and degree of enzyme deficiency determine the severity of the phenotype in most cases. Mothers of affected infants are expected to be carriers of the mutant gene unless a de novo pathogenic variant has occurred. A mother who gave birth to two affected male offspring was found to have a normal

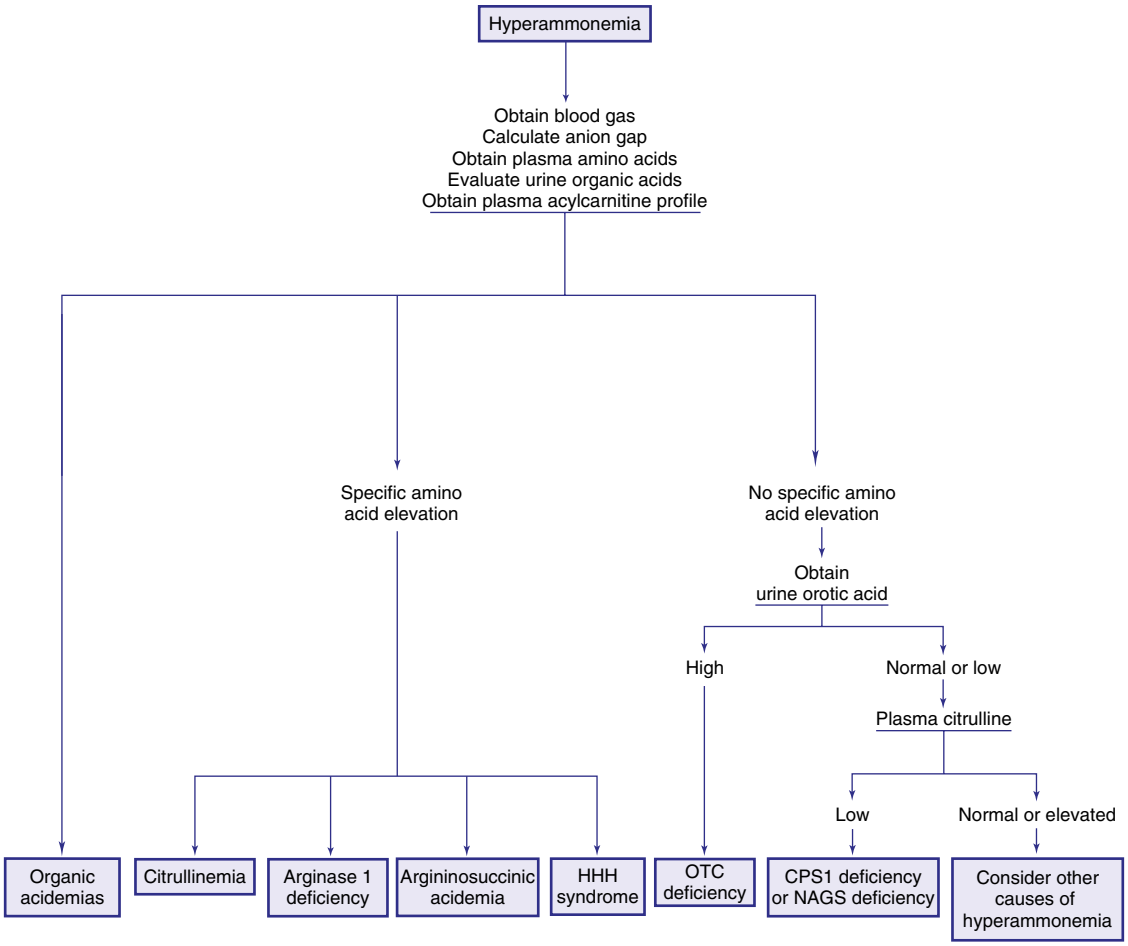

Fig. 105.14 Clinical algorithmic approach to a newborn infant with symptomatic hyperammonemia. CPS1, Carbamoyl phosphate synthetase 1; HHH syndrome, hyperornithinemia-hyperammonemia-homocitrullinemia; NAGS, *N*-acetylglutamate synthetase; OTC, ornithine carbamoyltransferase.

genotype, suggesting that gonadal mosaicism can be seen. As many as 20% of affected patients demonstrate normal results by Sanger sequencing, perhaps because the pathogenic variant involves copy number variants, deep intronic regions, or a promoter sequence. Copy number variants can be evaluated using a chromosomal microarray, and if positive, a contiguous gene deletion should be considered. If the molecular diagnostic approach is negative, a liver biopsy may be indicated. Prenatal diagnosis is feasible by analysis of DNA in amniocytes or chorionic villus samples. Increase in urinary excretion of orotidine after an allopurinol loading test can identify female carriers. Mild cerebral dysfunction may be present in asymptomatic female carriers. The importance of a detailed family history cannot be overemphasized. A history of migraine or protein aversion is common in maternal female relatives of the proband. Indeed, careful scrutiny of the family history may reveal a pattern of unexplained deaths in male newborns in the maternal lineage.

Treatment of acute hyperammonemic attacks and the long-term therapy of the condition are as outlined earlier. For enteral use, citrulline is used in place of arginine for patients with OTC deficiency. Liver transplantation is a surgical treatment option for patients with severe OTC deficiency.

CITRULLINEMIA

Two clinically and genetically distinct forms of citrullinemia have been described. The classic form (**type I**) is caused by the deficiency of the ASS enzyme. Citrullinemia **type II** is caused by the deficiency of a mitochondrial transport protein called *citrin*. (See Figs. 105.13 and 105.14.)

Citrullinemia Type I (Argininosuccinate Synthetase Deficiency, Classic Citrullinemia)

This condition is caused by the deficiency of ASS (see Fig. 105.13) and has variable clinical manifestations depending on the degree of the enzyme deficiency. Two major forms of the condition have been identified. The **severe** or **neonatal form**, which is most common, appears in the first few days of life with signs and symptoms of hyperammonemia (see earlier). In the **subacute or mild form**, clinical findings such as failure to thrive, frequent vomiting, developmental delay, and dry, brittle hair appear gradually after 1 year of age. Acute hyperammonemia, triggered by an intercurrent catabolic state, may bring the diagnosis to light.

Laboratory findings are similar to those found in patients with OTC deficiency, except that the plasma citrulline concentration is greatly elevated (50-100 times the reference range) (see Fig. 105.14). Urinary excretion of orotic acid is moderately increased; crystalluria may also occur as a result of precipitation of orotates. The diagnosis is confirmed by DNA analysis of *ASS1* or, less frequently, by an assay of enzyme activity in cultured fibroblasts. Prenatal diagnosis is feasible with an enzyme assay in cultured amniotic cells or by DNA analysis of cells obtained from chorionic villus biopsy.

Treatment of acute hyperammonemic attacks and long-term therapy are outlined earlier (see Table 105.6). Plasma concentration of citrulline remains elevated at all times and may increase further after administration of arginine. Patients can do well on a protein-restricted diet in conjunction with sodium benzoate, phenylbutyrate, and arginine therapy. Mild to moderate cognitive impairment is a common sequela, even in a well-treated patient.

Citrullinemia is an autosomal recessive condition caused by biallelic pathogenic variants in *ASS1*. The majority of patients are compound heterozygotes for two different alleles. The prevalence of the condition is 1 in 250,000 live births. The recent introduction of neonatal screening for urea cycle defects has shown that some affected patients are ostensibly asymptomatic even on a regular diet. Long-term follow-up is needed to be certain that these individuals do not sustain neurologic sequelae.

Citrin Deficiency (Citrullinemia Type II)

Citrin (aspartate-glutamate carrier protein) is a mitochondrial transporter encoded by *SLC25A13*. One of this protein's functions is to transport aspartate from mitochondria into cytoplasm and replenish the cytosolic aspartate pool required for converting citrulline to argininosuccinic acid (see Fig. 105.13). If aspartate is unavailable to the cytoplasmic component of the urea cycle, urea will not be formed at a normal rate, and citrulline will accumulate. ASS activity is diminished in the liver of these patients, but no pathogenic variant in the *ASS1* gene has been found. It is postulated that citrin deficiency interferes with translation of the messenger RNA for the ASS enzyme in the liver. The condition initially was reported in Japan (regional prevalence ~1 in 20,000), but many non-Japanese patients have been since identified (worldwide prevalence between 1 in 100,000 and 1 in 230,000). Biallelic pathogenic variants in *SLC25A13* have been associated with three clinical forms of citrin deficiency.

Neonatal Intrahepatic Cholestasis (Citrullinemia Type II, Neonatal Form)

Clinical and laboratory manifestations, which usually start before the infant's first birthday, include cholestatic jaundice with mild to moderate direct (conjugated) hyperbilirubinemia, marked hypoproteinemia, and clotting dysfunction (increased prothrombin time and partial thromboplastin time); increased serum γ-glutamyltransferase and alkaline phosphatase activities; and liver transaminases are usually normal. Plasma concentrations of ammonia and citrulline are usually normal, but moderate elevations have been reported. There may be increases in plasma concentrations of methionine, tyrosine, alanine, and threonine. Elevated levels of serum galactose have been found, even though the enzymes of galactose metabolism are normal. The reason for hypergalactosemia is not clear. Marked elevation in the serum level of α-fetoprotein is also present. These findings resemble those of tyrosinemia type I, but unlike the latter condition, urinary excretion of succinylacetone is not elevated (see Chapter 105.2). Liver biopsy shows fatty infiltration, cholestasis with dilated canaliculi, and a moderate degree of fibrosis. The condition is usually self-limiting, and the majority of infants recover spontaneously by 1 year of age with supportive and symptomatic treatment. Hepatic failure requiring liver transplantation has occurred in a few cases. Although the condition is commonly seen in Japan, the diagnosis should be considered in any case of unexplained neonatal hepatitis with cholestasis. Data on the long-term prognosis and the natural history of the condition are limited; development into the adult form of the condition after several years of a seemingly asymptomatic hiatus has been observed.

Failure to Thrive and Dyslipidemia Due to Citrin Deficiency

Some patients can present between 1 and 10 years of age with peculiar dietary habits favoring high-protein and high-lipid foods. It is accompanied by poor appetite, hypoglycemic episodes, and failure to thrive. Laboratory evaluation may reveal mild hyperammonemia, hypertriglyceridemia, elevated low-density lipoprotein (LDL) cholesterol, and low high-density lipoprotein (HDL) cholesterol levels. This presentation of citrin deficiency in children is often considered a prodromal form of the adult form of citrullinemia type II.

Citrullinemia Type II, Adult Form (Adult-Onset Citrullinemia; Citrullinemia Type II, Mild Form)

Without prior molecular diagnosis or family history, individuals affected by this form of citrullinemia type II (CTLN2) are often identified acutely. A previously healthy individual may present with nonspecific neuropsychiatric symptoms such as disorientation, delirium, delusion, aberrant behavior, tremors, and psychosis. Moderate degrees of hyperammonemia and hypercitrullinemia are present. The age at onset is usually between 20 and 40 years but can happen at any point after age 11 years. Patients who recover from the first episode may have recurrent attacks. Pancreatitis, hyperlipidemia, and hepatoma are major complications among the survivors. Medical **treatment** of CTLN2 has been mostly ineffective to prevent future attacks. A diet enriched in protein and lipids helps restore cytosolic aspartate and stimulate ureagenesis. In confirmed citrin deficiency, a low-protein/high-carbohydrate diet should be avoided. Although liver transplantation appears to be effective in preventing future episodes of hyperammonemia, enteral supplementation with pyruvate, arginine, and medium-chain triglycerides can be tried first to help prevent hyperammonemic episodes and improve growth.

ARGININOSUCCINATE LYASE DEFICIENCY (ARGININOSUCCINIC ACIDURIA)

The severity of the clinical and biochemical manifestations varies considerably (see Figs. 105.13 and 105.14). In the severe form of ASL deficiency, severe hyperammonemia (see earlier) can develop in the first few days of life, and without treatment affected infants can perish. The clinical course of ASL deficiency in patients who survive the initial acute episode is often characterized by intellectual disability, failure to thrive, hypertension, gallstones, liver fibrosis, and hepatomegaly. A common finding in untreated patients is dry and brittle hair (**trichorrhexis nodosa**), although this finding is relatively nonspecific. Acute attacks of severe hyperammonemia may occur during a catabolic state triggered by infections, trauma, or dietary indiscretion. Laboratory findings include hyperammonemia, moderate elevations in liver enzymes, nonspecific increases in plasma levels of glutamine and alanine, a moderate increase in plasma levels of citrulline (less than in citrullinemia), and a marked increase in the concentration of argininosuccinic acid in plasma, urine, and CSF. The CSF levels are usually higher than those in plasma. The enzyme is present in erythrocytes, the liver, and cultured fibroblasts. **Prenatal diagnosis** is possible by identification of biallelic pathogenic variants in the *ASL* gene or, rarely, by measurement of the enzyme activity in cultured amniotic cells. Argininosuccinic acid is also elevated in the amniotic fluid of affected fetuses.

Treatment of acute hyperammonemic attacks and the long-term therapy of the condition are outlined earlier in this chapter. Intellectual disability, persistent hepatomegaly with mild increases in liver enzymes, and bleeding tendencies as a result of abnormal clotting factors are common sequelae. ASL deficiency is an autosomal recessive disorder with a prevalence of about 1 in 220,000 live births. Early detection is achieved through mass screening of newborn infants.

ARGINASE 1 DEFICIENCY (HYPERARGININEMIA)

Arginase 1 deficiency is an autosomal recessive condition caused by biallelic pathogenic variants in *ARG1* (see Figs. 105.13 and 105.14). Two genetically distinct arginases are present in humans. Arginase 1 (ARG1) is a cytosolic enzyme and is expressed in the liver and can be found in erythrocytes. Arginase 2 (ARG2) is expressed in renal and brain mitochondria. The role of ARG2 is not well understood; its activity in patients with argininemia appears to have no protective effect.

Clinical manifestations of arginase 1 deficiency are somewhat different from other urea cycle enzyme defects, although acute neonatal hyperammonemia presenting with intractable seizures, cerebral edema, and death has also been reported. Outside of the neonatal period, the onset of arginase 1 deficiency often is insidious. The infant can remain asymptomatic in the first few months or years of life. A **progressive spastic diplegia** with scissoring of the lower extremities, choreoathetotic movements, loss of developmental milestones, and failure to thrive in a previously normal infant may suggest the diagnosis. Some children were treated for cerebral palsy before arginase 1 deficiency was confirmed. Intellectual disability is progressive; seizures are common, but episodes of severe hyperammonemia are not as frequent as in the more proximal urea cycle defects. Hepatomegaly may be present.

Laboratory evaluation reveals marked elevations of arginine in plasma and CSF (see Fig. 105.14). Urinary orotic acid can be increased. Determination of amino acids in plasma is a critical step in the diagnosis of argininemia. Guanidino compounds (α-keto-guanidinovaleric acid and α-keto-argininic acid) can be markedly increased in urine. The diagnosis is secured by identification of biallelic pathogenic variants in *ARG1* or, rarely, by assaying arginase activity in erythrocytes.

Treatment consists of a low-protein diet at the age-appropriate RDA. The dietary composition and daily intake of protein should be monitored by frequent plasma amino acid determinations. Supplementation with arginine in ARG1-deficient patients is contraindicated. Sodium benzoate or sodium phenylbutyrate is also effective in controlling hyperammonemia and lowering plasma arginine levels. Liver transplantation has produced promising results, but experience with long-term outcome is limited. Early detection is feasible through mass screening of newborn infants.

TRANSIENT HYPERAMMONEMIA OF THE NEWBORN

The blood concentration of ammonia in a full-term infant can be as high as 100 μmol/L, or two to three times greater than that of the older child or adult. Blood levels approach the adult normal values after a few weeks of life (see Fig. 105.14). Plasma ammonia levels greater than 100 μmol/L should prompt additional diagnostic steps to evaluate for possible genetic causes of hyperammonemia. Infrequently, **severe transient hyperammonemia** can be observed in infants whose diagnostic workup reveals no biochemical abnormalities implicating genes encoding components of the urea cycle. The majority of affected infants are premature and have mild respiratory distress syndrome. Hyperammonemic coma can develop within 2-3 days of life, and the infant may succumb to the disease if treatment is not started immediately. Laboratory studies reveal marked hyperammonemia (plasma ammonia as high as 4,000 μmol/L) with moderate increases in plasma levels of glutamine and alanine. Plasma concentrations of urea cycle intermediate amino acids are usually normal except for citrulline, which may be moderately elevated. The cause of the disorder is unknown. Urea cycle enzyme activities are normal. **Treatment** of hyperammonemia should be initiated promptly and continued vigorously. Recovery without sequelae has been reported, and hyperammonemia does not recur even with a normal-protein diet.

DISORDERS OF ORNITHINE METABOLISM

Ornithine, a key intermediate of the urea cycle, is not incorporated into natural proteins. Rather, it is generated in the cytosol from arginine and must be transported into mitochondria, where it becomes a substrate in reactions catalyzed by OTC forming citrulline. Excess ornithine is catabolized by two enzymes: ornithine aminotransferase, a mitochondrial enzyme converting ornithine to a proline precursor, and ornithine decarboxylase, which resides in the cytosol and converts ornithine to putrescine (see Fig. 105.13). Two genetic disorders feature **hyperornithinemia**: gyrate atrophy of the retina and hyperammonemia-hyperornithinemia-homocitrullinemia (HHH) syndrome.

Gyrate Atrophy of the Retina and Choroid

This rare, autosomal recessive disorder is caused by biallelic pathogenic variants in *OAT* leading to the deficient activity of ornithine aminotransferase (see Fig. 105.13). Approximately 30% of the reported cases are from Finland. Clinical manifestations may include hyperammonemia in the first months of life in some patients. Findings that define the phenotype of ornithine aminotransferase deficiency include night blindness, myopia, loss of peripheral vision, and posterior subcapsular cataracts. These eye changes start between 5 and 10 years of age and progress to complete blindness by the fourth decade of life. Atrophic lesions in the retina resemble cerebral gyri. These patients usually have normal intelligence. Besides the characteristic 10- to 20-fold increase in plasma levels of ornithine (400-1,400 μmol/L), plasma levels of glutamate, glutamine, lysine, creatine, and creatinine can be moderately decreased. Some patients show partial improvement with high doses of pyridoxine. An arginine-restricted diet in conjunction with supplemental lysine, proline, and creatine has been successful in reducing plasma ornithine concentration and has produced some clinical improvement.

Hyperammonemia-Hyperornithinemia-Homocitrullinemia Syndrome

Biallelic pathogenic variants in *SLC25A15* result in an autosomal recessive disorder, HHH syndrome. This defect of the ornithine transport system leads to accumulation of ornithine in the cytosol and a depletion of this amino acid in mitochondria. The former causes hyperornithinemia, and the latter results in disruption of the urea cycle and hyperammonemia (see Fig. 105.13). Homocitrulline is presumably formed through condensation of mitochondrial carbamoyl phosphate with lysine. Clinical manifestations of hyperammonemia can develop shortly after birth or may be delayed until adulthood. Acute episodes of hyperammonemia manifest as refusal to feed, vomiting, and lethargy; coma may occur during infancy. Progressive neurologic signs, such as lower limb weakness, increased deep tendon reflexes, spasticity, clonus, seizures, and varying degrees of psychomotor retardation may develop if the condition remains undiagnosed. No ocular findings have been observed in these patients. Laboratory findings reveal marked increases in plasma levels of ornithine and homocitrulline in addition to hyperammonemia (see Fig. 105.14). Acute episodes of hyperammonemia should be treated promptly (see earlier). Restriction of protein intake improves hyperammonemia. Oral supplementation with arginine (or citrulline) has produced clinical improvement in some patients.

CONGENITAL GLUTAMINE DEFICIENCY

Glutamine is synthesized endogenously from glutamate and ammonia by a ubiquitously expressed enzyme, glutamine synthetase (see Fig. 105.11). Glutamine is known to be involved in several important functions, including detoxification of ammonia. Deficiency of this enzyme, resulting in glutamine deficiency, has been reported in three infants from three unrelated families. All affected infants manifested multiorgan involvement, including brain malformations (abnormal gyrations, hypomyelination), facial abnormalities (broad nasal root, low-set ears), hypotonia, and seizures at birth. Two of the patients died from multiorgan failure (respiratory and heart failure) in the neonatal period. One child was alive at 3 years of age with severe developmental delay. Glutamine was absent in plasma, urine, and CSF, but plasma levels of glutamic acid were normal. Congenital glutamine deficiency is an autosomal recessive condition caused by biallelic pathogenic variants in *GLUL*.

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105.13 Histidine

Oleg A. Shchelochkov and Charles P. Venditti

Histidine is degraded through the urocanic acid pathway to glutamic acid. Several genetic biochemical aberrations involving the degradative pathway of histidine have been reported, but the clinical significance of elevated histidine levels has not been established.

Decarboxylation of histidine by histidine decarboxylase produces histamine. Deficiency of this enzyme has been implicated in the familial form of **Tourette syndrome** (see Chapter 105.11).

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105.14 Lysine

Oleg A. Shchelochkov and Charles P. Venditti

Lysine is catabolized through two pathways. In the first pathway, lysine is condensed with α-ketoglutaric acid to form saccharopine. Saccharopine is then catabolized to α-aminoadipic semialdehyde and glutamic acid. These first two steps are catalyzed by α-aminoadipic semialdehyde synthase, which has two activities: lysine-ketoglutarate reductase and saccharopine dehydrogenase (Fig. 105.15). In the second pathway, lysine is first transaminated and then condensed to its cyclic forms: pipecolic acid and piperideine-6-carboxylic acid **(P6C)**. P6C and its linear form, α-aminoadipic semialdehyde, are oxidized to α-aminoadipic acid by the enzyme **antiquitin**. This is the major pathway for D-lysine in the body and for L-lysine in the brain.

Hyperlysinemia-saccharopinuria, α-aminoadipic, and α-ketoadipic acidemia are biochemical conditions caused by inborn errors of lysine degradation. Individuals with these conditions are usually asymptomatic.

PYRIDOXINE–DEPENDENT EPILEPSY (PDE)

Pyridoxal 5′-phosphate **(P5P)**, the active form of pyridoxine (vitamin B_6), is the cofactor for many enzymes, including those involved in the metabolism of neurotransmitters. Intracellular P5P deficiency in the brain may result in a seizure disorder that is refractory to common anticonvulsant agents but is responsive to high doses of pyridoxine. These pyridoxine-responsive phenotypes are seen in the following genetic metabolic conditions.

Antiquitin (α-Aminoadipic Semialdehyde Dehydrogenase) Deficiency

This is the most common cause of PDE. A deficiency of antiquitin (encoded by *ALDH7A1*) results in accumulation of P6C in brain tissue (see Fig. 105.15). P6C reacts with P5P and renders it inactive. Large doses of pyridoxine are therefore needed to overcome this inactivation. The condition is inherited as an autosomal recessive trait.

Pyridox(am)ine 5′-Phosphate Oxidase (PNPO) Deficiency

PNPO deficiency clinically overlaps with antiquitin deficiency. PNPO-deficient patients often present with neonatal-onset seizures, developmental delays, spastic tetraplegia, and nonspecific findings on brain imaging (delayed myelination, cerebral atrophy, and abnormal signals in the basal ganglia). Developmental regression, optic disc pallor, and retinopathy have been reported infrequently. Plasma and CSF amino acid analysis may reveal elevated glycine, prompting evaluation for NKH (see Chapter 105.7), leading to a delay in initiating treatment with P5P. A CSF neurotransmitter assay revealed inconsistent changes in the levels of 3-*O*-methyldopa, homovanillic acid, and 5-hydroxyindoleacetic acid. A normal CSF level of P5P was reported in one patient, suggesting that a therapeutic trial with P5P and molecular analysis may be a prudent strategy in some patients irrespective of the CSF studies. The lowest effective dose of P5P should be used to avoid toxicity. The disorder is caused by biallelic pathogenic variants in *PNPO*.

Sulfite Oxidase Deficiency and Molybdenum Cofactor Deficiency

In this rare condition (see Chapter 105.4), accumulation of sulfites causes inhibition of enzymatic activity of antiquitin and accumulation of P6C, which in turn causes inactivation of P5P and vitamin B_6 dependency.

Hyperprolinemia Type II

In this condition, accumulation of P5C in brain tissue can also cause inactivation of P5P, leading to pyridoxine dependency (see Chapter 105.9 and Fig. 105.9).

Hypophosphatasia

P5P is the main circulating form of pyridoxine. Alkaline phosphatase (ALP) is required for dephosphorylation of P5P to generate free pyridoxine, which is the only form of vitamin B_6 that can cross the BBB and enter the brain cells. Pyridoxine is rephosphorylated

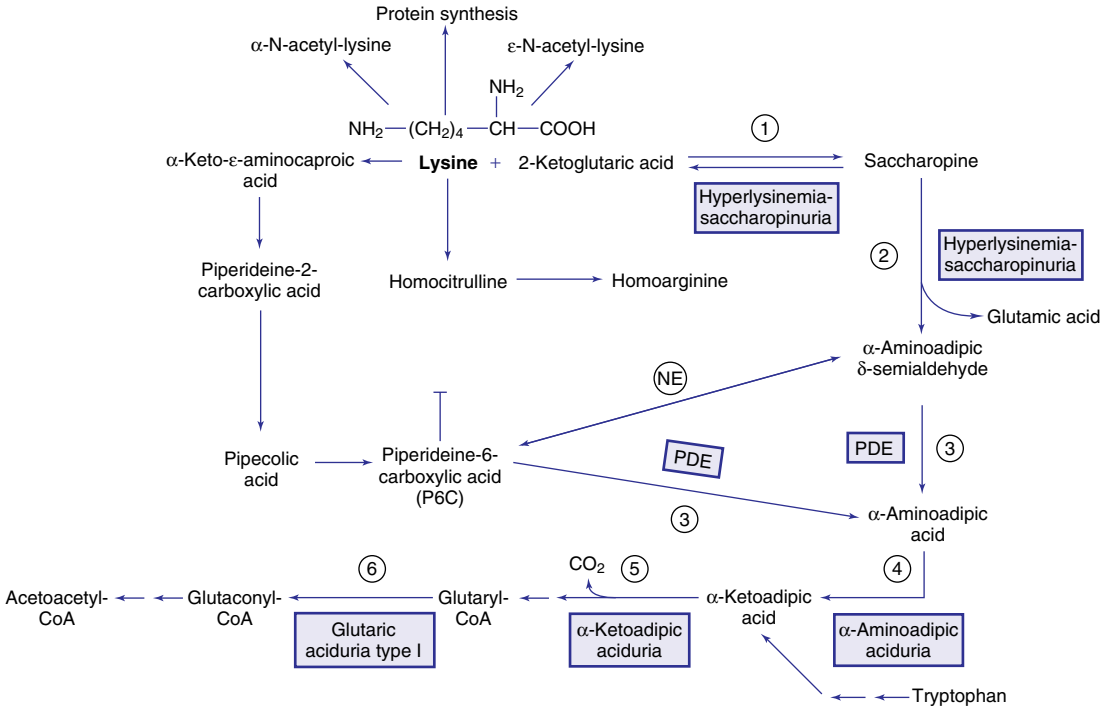

Fig. 105.15 Pathways in the metabolism of lysine. **Enzymes:** *(1)* Lysine ketoglutarate reductase, *(2)* saccharopine dehydrogenase, *(3)* α-aminoadipic semialdehyde/piperidine-6-carboxylic acid (P6C) dehydrogenase (antiquitin), *(4)* α-aminoadipic acid transferase, *(5)* α-ketoadipic acid dehydrogenase, *(6)* glutaryl-CoA-dehydrogenase. NE, Nonenzymatic; PDE, pyridoxine-dependent epilepsy.

intracellularly to form P5P. In the infantile form of hypophosphatasia, P5P cannot be dephosphorylated to free pyridoxine because of the marked deficiency of tissue-nonspecific ALP. This results in deficiency of pyridoxine in the brain and PDE (see Chapters 611 and 724).

The main **clinical manifestation** of PDE caused by antiquitin deficiency is generalized seizures, which usually occur in the first days of life and are unresponsive to conventional anticonvulsant therapies. Some mothers of affected fetuses report abnormal intrauterine fluttering movements. The seizures are usually tonic-clonic in nature but can be almost any type. Other manifestations such as dystonia, respiratory distress, and abdominal distention with vomiting, hepatomegaly, hypoglycemia, and hypothermia may be present. Learning problems and speech delay are common sequelae. Late-onset forms of the condition (as late as 5 years of age) have been reported. Consequently, a trial with vitamin B_6 is recommended in any infant with intractable convulsions (see Chapters 611.04 and 611.06).

Laboratory findings show increased concentrations of α-aminoadipic semialdehyde and pipecolic acid in the CSF, plasma, and urine. EEG abnormalities may normalize after treatment. Neuroimaging may be normal, but cerebellar and cerebral atrophy, periventricular hyperintensity, intracerebral hemorrhage, and hydrocephalus have been reported.

Treatment with vitamin B_6 (50-100 mg/day) usually results in a dramatic improvement of both seizures and the EEG abnormalities. High doses of pyridoxine can result in peripheral neuropathy, and doses >500 mg/day should be avoided. The pyridoxine dependency, and thus the therapy, are lifelong. The therapeutic benefit of a lysine-restricted, L-arginine-enriched diet is being evaluated.

Glutaric Aciduria Type 1 (Glutaryl-CoA Dehydrogenase Deficiency)

Glutaric acid is an intermediate in the degradation of lysine (see Fig. 105.15), hydroxylysine, and tryptophan. Glutaric aciduria type 1, a disorder caused by a deficiency of glutaryl-CoA dehydrogenase, should be differentiated from glutaric aciduria type 2, a distinct clinical and biochemical disorder caused by defects in the mitochondrial electron transport chain (see Chapter 106.1).

Glutaric aciduria type 1 is an autosomal recessive disorder caused by biallelic loss-of-function variants in *GCDH*. Its prevalence is estimated at 1 in 100,000 live births worldwide. The condition is more prevalent in some ethnic populations (e.g., Canadian Oji-Cree Indians, Irish Travelers, Black South Africans, Swedes, and the Old Order Amish population in the United States). A high prevalence of known pathogenic variants in specific ethnic populations can enable a cost-effective molecular evaluation and counseling. **Prenatal diagnosis** can be accomplished by demonstrating increased concentrations of glutaric acid in amniotic fluid, by assay of the enzyme activity in amniocytes or chorionic villus samples, or by identification of the known pathogenic variants in *GCDH*.

Clinical Manifestations

Macrocephaly is a common, but nonspecific, finding in patients with glutaric aciduria type 1. It develops in the first year of life but can also be present at birth and precede the onset of neurologic manifestations. Some affected infants may also show subtle neurologic symptoms, such as delayed onset of motor milestones, irritability, and feeding problems, during this seemingly asymptomatic period. The onset of the condition is usually heralded by **acute encephalopathic findings**, such as loss of normal developmental milestones (head control, rolling over, or sitting), seizures, generalized rigidity, opisthotonos, choreoathetosis, and dystonia caused by acute striatal injury. These symptoms may occur suddenly in an apparently normal infant after a minor infection. Brain imaging reveals increased extraaxial (particularly frontal) fluid with stretched bridging veins, striatal lesions, dilated lateral ventricles, cortical atrophy (mainly in the frontotemporal region), and fibrosis. Recovery from the first attack usually occurs slowly, and some residual neurologic abnormalities may persist, especially dystonia and choreoathetosis. Without treatment, additional acute attacks resembling the first can occur during subsequent episodes of intercurrent infections or catabolic states. In some patients, these signs and symptoms may develop gradually in the first few years of life. Hypotonia and choreoathetosis may gradually progress into rigidity and dystonia **(insidious form)**. Acute episodes of metabolic decompensation with vomiting, ketosis, seizures, and coma also occur in this form after infection or other catabolic states. Without treatment, death may occur in the first decade of life during one of these episodes. Affected infants are prone to development of subdural hematoma and retinal hemorrhage after minor falls and head traumas. This can be misdiagnosed as child abuse. The intellectual abilities usually remain relatively normal in most patients.

Laboratory Findings

During acute episodes, mild to moderate metabolic acidosis and ketosis may occur. Hypoglycemia, hyperammonemia, and elevations of serum transaminases are seen in some patients. High concentrations of glutaric acid are usually found in the urine, blood, and CSF. 3-Hydroxyglutaric acid may also be present in the body fluids. The acylcarnitine profile shows elevated glutarylcarnitine (C5DC) in blood and urine. Plasma concentrations of amino acids are usually within normal limits. Laboratory findings may be unremarkable between attacks. Glutaric aciduria type 1 can be identified on the newborn screen by measuring glutarylcarnitine (C5DC) levels in blood spots. The sensitivity of this screening method depends on the cutoff value used by a newborn screen program, and some patients may not be detected. For example, a subset of patients with glutaric aciduria type 1 can present with normal plasma and urinary levels of glutaric acid and variably elevated plasma glutarylcarnitine. This type of glutaric aciduria type 1, referred to as a "low-excretor" phenotype, carries the same risk of developing brain injury as in a "high-excretor" phenotype. In some low-excreting patients, glutaric acid is elevated only in the CSF. Urinary glutarylcarnitine appears to be a more sensitive screening method to identify affected low-excreting patients. Molecular analysis of *GCDH* can aid in identifying patients with a low-excretor phenotype associated with specific pathogenic variants (e.g., p.M405V, p.V400M, p.R227P). We recommend performing molecular analysis of *GCDH* or glutaryl-CoA dehydrogenase enzyme activity in any child presenting with unexplained progressive dystonia and dyskinesia.

Treatment

Patients require a lysine- and tryptophan-restricted diet while meeting physiologic requirements for protein, micronutrients, and vitamins. Increased dietary arginine may decrease cellular uptake of lysine and decrease the endogenous formation of glutaryl-CoA. Patients should be routinely evaluated for lysine and tryptophan deficiency by monitoring plasma amino acids and growth. L-Carnitine supplementation (50-100 mg/kg/day orally) is recommended in all cases. Emergency treatment during acute illness, including temporary cessation of protein intake for 24 hours, replacement of lost calories using carbohydrates or lipids, IV L-carnitine, IV dextrose, prompt treatment of infection, and control of fever is critical to decreasing the risk of striatal injury. All patients should be provided with an emergency letter describing the underlying diagnosis, recommended evaluation, and treatment. Early diagnosis through newborn screening with prevention and aggressive treatment of intercurrent catabolic states (infections) can help minimize striatal injury and ensure a more favorable prognosis. Patients with movement disorder and spasticity may require treatment with baclofen, diazepam, trihexyphenidyl, and injectable botulinum toxin A.

LYSINURIC PROTEIN INTOLERANCE (LPI)

This rare autosomal recessive disorder is caused by pathogenic variants in *SLC7A7* leading to impaired function of a protein transporting the cationic amino acids lysine, ornithine, and arginine in the intestine and kidneys. A deficiency of the transporter protein (Y+L amino acid transporter 1) in this condition causes multisystem manifestations, which often start with gastrointestinal symptoms. The transport defect in this condition resides in the basolateral (antiluminal) membrane of enterocytes and renal tubular epithelia. This explains the observation that cationic amino acids are unable to cross these cells even when administered as dipeptides. Lysine in the form of dipeptide crosses the luminal membrane of the enterocytes but hydrolyzes to free lysine in the cytoplasm. Free lysine, unable to cross the basolateral membrane of the cells, can diffuse back into the lumen leading to gastrointestinal symptoms.

Refusal to feed, nausea, aversion to protein, vomiting, and mild diarrhea, which may result in failure to thrive, wasting, and hypotonia, can be seen shortly after birth. Breastfed infants usually remain asymptomatic until soon after weaning, possibly because of the switch to a higher-protein solid foods. Episodes of hyperammonemia caused by the depletion of ornithine and arginine in the urea cycle may occur after ingestion of a high-protein meal. Mild to moderate hepatosplenomegaly, osteoporosis, sparse brittle hair, thin extremities with moderate centripetal adiposity, and growth retardation are common physical findings in patients whose condition has remained undiagnosed. Neurocognitive status is usually normal, but moderate intellectual disability has been observed in some patients.

Progressive interstitial pneumonitis with bouts of acute exacerbation often occurs and often progresses to severe alveolar proteinosis. Clinical manifestations include progressive exertional dyspnea, fatigue, cough, diminished breath sound, and inspiratory rales; cyanosis may develop in older patients. Some LPI patients have remained undiagnosed until the appearance of pulmonary manifestations. Radiographic evidence of pulmonary fibrosis has been observed in up to 65% of patients without clinical manifestations of pulmonary involvement.

Renal involvement is manifested initially by proteinuria, hematuria, and elevation of serum creatinine, which may progress to end-stage renal failure. Renal tubular involvement with laboratory findings of renal Fanconi syndrome may also be present. Renal biopsy reveals pathologic findings consistent with glomerulonephritis and tubulointerstitial nephritis. Hematologic findings of anemia, leukopenia, thrombocytopenia, and elevated ferritin may also be present.

Other organs are frequently involved. A condition resembling **hemophagocytic lymphohistiocytosis/macrophage activation syndrome** has been reported. Immunologic abnormalities (impaired lymphocyte function, abnormalities in immune globulins, hypocomplementemia) and acute pancreatitis are frequent features of LPI.

Pregnancies in affected mothers have been complicated by anemia, thrombocytopenia, toxemia, and bleeding, but offspring appear healthy at birth.

Laboratory findings may reveal hyperammonemia and an elevated concentration of urinary orotic acid, which develop after high-protein feeding. Plasma concentrations of lysine, arginine, and ornithine are usually mildly decreased, but urinary levels of these amino acids, especially lysine, are greatly increased. The pathogenesis of hyperammonemia is likely related to the depletion of urea cycle intermediates caused by poor absorption and the increased renal loss of ornithine and arginine. Plasma concentrations of alanine, glutamine, serine, glycine, and proline are usually increased. Anemia, increased serum levels of ferritin, LDH, thyroxine-binding globulin, hypercholesterolemia, and hypertriglyceridemia are common findings often leading to the workup for hemophagocytic lymphohistiocytosis. This condition should be differentiated from hyperammonemia caused by urea cycle defects (see Chapter 105.12), especially in heterozygous females with OTC deficiency, in whom increased urinary excretion of lysine, ornithine, and arginine is not seen.

Treatment with a low-protein diet providing the RDA of protein and supplemented with oral citrulline (50-100 mg/kg/day) can produce biochemical and clinical improvements. Episodes of hyperammonemia should be treated promptly (see Chapter 105.12). Supplementation with lysine (10-30 mg/kg/day) given in small and frequent doses helps improve plasma levels. The dose of lysine should be titrated down if patients develop abdominal pain and diarrhea. Treatment with high doses of prednisone has been effective in the management of acute pulmonary complications in some patients. **Bronchopulmonary lavage** is the treatment of choice for patients with alveolar proteinosis. The condition is more prevalent in Finland and Japan, where the prevalence is 1 in 60,000 and 1 in 57,000 live births, respectively.

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105.15 *N*-Acetylaspartic Acid (Canavan Disease)

Reuben K. Matalon[†] and Dena R. Matalon

N-Acetylaspartic acid **(NAA)**, a derivative of aspartic acid, is synthesized in the brain and is found in a high concentration similar to glutamic acid. NAA has multiple functions, such as serving as an acetate reservoir for myelin synthesis, as well as being an organic osmolyte that helps regulate cerebral osmolality. However, the complete function of NAA is not yet fully understood. **Aspartoacylase** (ASPA) cleaves the *N*-acetyl group from NAA. A deficiency of aspartoacylase leads to **Canavan disease**, a severe **leukodystrophy** characterized by excessive excretion of NAA and spongy degeneration of the white matter of the brain. Canavan disease is an autosomal recessive disorder and is more prevalent in individuals of Ashkenazi Jewish descent than in other ethnic groups. The defective gene for Canavan disease *(ASPA)* can be tested in patients, family members, and at-risk populations.

ETIOLOGY AND PATHOLOGY

Deficiency of the enzyme aspartoacylase due to pathogenic variants in the *ASPA* gene leads to NAA accumulation in the brain, especially in the white matter, and massive urinary excretion. Excessive amounts of NAA are also present in the blood and CSF. Brain biopsies of patients with Canavan disease show spongy degeneration of the myelin fibers, astrocytic swelling, and elongated mitochondria. There is striking vacuolization and astrocytic swelling in the white matter. Electron microscopy reveals distorted mitochondria. As the disease progresses, the ventricles enlarge because of cerebral atrophy.

CLINICAL MANIFESTATIONS

The severity of Canavan disease covers a wide spectrum. Infants usually appear normal at birth and may not manifest symptoms of the disease until 3-6 months of age, when they develop **progressive macrocephaly**, severe hypotonia, persistent head lag, and delayed milestones. As the disease progresses, there is spasticity, joint stiffness, and contractures. Optic atrophy and seizures subsequently develop. Feeding difficulties, poor weight gain, and gastroesophageal reflux may occur in the first year of life followed by swallowing deterioration that may require nasogastric feeding or permanent gastrostomy. In the past, most individuals died in the first decade of life, but with the advances in medical technology and improved supportive care, survival now extends to the second or third decade.

[†]Deceased

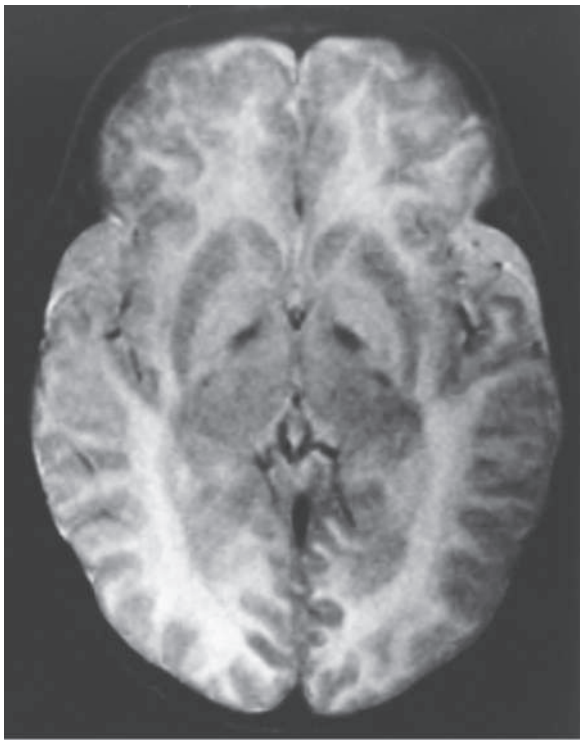

Fig. 105.16 Axial T2-weighted MRI of a 2-yr-old patient with Canavan disease. Extensive thickening of the white matter radiation is seen.

ATYPICAL CANAVAN DISEASE

Juvenile or **mild** Canavan disease is less common than **infantile** Canavan disease and is most prevalent in non-Ashkenazi Jews. Affected individuals with juvenile Canavan disease usually present with mild speech and motor delay and may have **retinitis pigmentosa**. The other typical features of Canavan disease are usually not present. These children have moderately increased urinary excretion of NAA to suggest Canavan disease. Brain MRI demonstrates increased signal intensity in the basal ganglia rather than global white matter disease, sometimes leading to confusion with mitochondrial disease.

DIAGNOSIS

In a typical individual with Canavan disease, brain MRI shows diffuse white matter degeneration, primarily in the cerebral hemispheres, with less involvement of the cerebellum and brainstem (Fig. 105.16). Repeated evaluations may be required. Magnetic resonance spectroscopy (MRS) can be done to show the high peak of NAA, which strongly suggests Canavan disease. The diagnosis can also be established by finding elevated amounts of NAA in the urine or blood. NAA is found in only trace amounts (24 ± 16 μmol/mmol creatinine) in the urine of unaffected individuals, whereas its concentration is in the range of 1,440 ± 873 μmol/mmol creatinine in individuals with Canavan disease. High levels of NAA can also be detected in plasma, CSF, and brain tissue. Aspartoacylase enzyme analysis from fibroblasts is often used to confirm the diagnosis but is not necessary. The activity of aspartoacylase in the fibroblasts of obligate carriers is half or less the activity found in normal individuals. Molecular analysis for variants in the *ASPA* gene is now recommended for all individuals in which Canavan disease is suspected.

The differential diagnosis of Canavan disease should include Alexander disease, another leukodystrophy associated with macrocephaly. Alexander disease is caused by a defect in the synthesis of glial fibrillary acidic protein, and the diagnosis can be ruled out by genetic testing of variants in the *GFAP* gene.

There are two predominant pathogenic variants leading to Canavan disease in the Ashkenazi Jewish population. The first is an amino acid substitution (p.Glu285Ala) in which glutamic acid is substituted for alanine. This pathogenic variant is the most frequent and encompasses 83% of mutant alleles in Ashkenazi Jewish individuals with Canavan disease. The second most common pathogenic variant is a change from tyrosine to a nonsense pathogenic variant, leading to a stop in the coding sequence (p.Tyr231*). This accounts for 13% of mutant Ashkenazi alleles. In the non-Jewish population, more diverse pathogenic variants have been observed, with the two variants common in the Ashkenazi Jewish population being rare. A different variant (p.Ala305Glu), the substitution of alanine for glutamic acid, accounts for 40% of mutant alleles in non-Jewish individuals. Over 70 pathogenic variants have been reported in the non-Jewish population. With Canavan disease, it is important to obtain a molecular diagnosis because this will lead to accurate counseling and prenatal guidance for the family. If the specific variants are not known, prenatal diagnosis relies on the NAA level in the amniotic fluid. In Ashkenazi Jewish individuals, the carrier frequency may be as high as 1:40, nearing that of Tay-Sachs disease. Carrier screening for Canavan disease is available. Genotype-phenotype studies and aspartoacylase expression and enzymatic activity help to prognosticate the severity of disease. Patients with juvenile or mild forms of Canavan disease have been compound heterozygotes with a mild pathogenic variant on one allele and a severe variant on the other allele. Mild variants include p.Tyr288Cys and p.Arg71His.

TREATMENT AND PREVENTION

Treatment for Canavan disease is supportive. After diagnosis, individuals should be monitored closely in terms of feeding and nutritional status, development, and risk for seizures. Medical intervention may be necessary such as antiepileptics, acetazolamide for increased intracranial pressures, and botulinum toxin injections for spasticity. Individuals benefit from early therapy interventions and special education programs to maximize developmental potential and communication. Genetic counseling, carrier testing, and prenatal diagnosis are the only current methods of prevention.

Studies of gene therapy using recombinant adeno-associated viruses (rAAVs) have shown some positive results in knockout mice models. Gene therapy attempts in children with Canavan disease have shown a lack of adverse events, some decrease in the brain elevation of *N*-acetylaspartic acid, improved seizure frequency, and slowing of the progression of brain atrophy. However, there was no improvement in long-term clinical status. Novel rAAV serotypes have been shown to better cross the **blood-brain barrier** and improved longevity of Canavan disease knockout mice, giving promise for further use of gene therapy in individuals with Canavan disease. **Enzyme-replacement therapy** with ASPA and pegylated ASPA is being studied in mice and has shown a decrease of NAA in the brain.

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Chapter 106

Defects in Metabolism of Lipids

106.1 Disorders of Mitochondrial Fatty Acid β-Oxidation

Jerry Vockley

Mitochondrial β-oxidation of fatty acids is an essential energy-producing pathway. It is particularly important during prolonged periods of reduced caloric intake such as fasting or gastrointestinal illness or increased energy expenditure during physiologic stress such as febrile illness. Under these conditions, the body switches from using predominantly *carbohydrate* to predominantly *fat* as its major fuel. Fatty acids are also important fuels for exercising skeletal muscle and are the preferred substrate for normal cardiac metabolism. In these tissues, fatty acids are completely oxidized to carbon dioxide and water. The end products of hepatic fatty acid oxidation are the ketone bodies 3-*hydroxybutyrate* and *acetoacetate*. These compounds cannot be oxidized by the liver but are exported to serve as important fuels in peripheral tissues, particularly the brain, where ketone bodies can partially substitute for glucose during periods of fasting.

Genetic defects have been identified in almost all the known steps in the fatty acid oxidation pathway; all are recessively inherited (Table 106.1).

Clinical manifestations characteristically involve tissues with a high β-oxidation flux, including liver, skeletal, and cardiac muscle, and are age dependent. The most common early presentation is an acute episode of life-threatening coma, hepatic encephalopathy, and hypoglycemia with or without hyperammonemia induced by a period of fasting or illness in the first 2-4 years of life. After about age 6 years, muscle symptoms predominate in the disorders of long-chain fatty acid oxidation, including myopathy, fatiguability, and recurrent rhabdomyolysis. The latter is often brought on by excess activity or exercise. Cardiomyopathy can occur at any age in individuals with long-chain defects and may be exacerbated with acute episodes of illness. Patients with fatty acid oxidation defects are often asymptomatic when well. Acutely presenting disease may be misdiagnosed as **Reye syndrome** or, if fatal, as **sudden unexpected infant death**. In some circumstances, clinical manifestations appear to arise from toxic effects of fatty acid metabolites rather than inadequate energy production. These circumstances include certain long chain fatty acid oxidation disorders (deficiencies of long chain 3-hydroxyacyl dehydrogenase **[LCHAD]**, carnitine palmitoyltransferase-IA **[CPT-IA]**, or mitochondrial trifunctional protein [**MTP**; also known as *TFP*]) in which the presence of a homozygous affected fetus increases the risk of a life-threatening illness in the heterozygote mother, resulting in **acute fatty liver of pregnancy** (AFLP) or **preeclampsia with HELLP** (hemolysis, elevated liver enzymes, low platelets) syndrome. The mechanism of these obstetric complications is likely accumulation of toxic intermediates. Malformations of the brain and kidneys have been described in severe deficiencies of electron transfer flavoprotein **(ETF)**, ETF dehydrogenase **(ETF-DH)**, and carnitine palmitoyltransferase-II **(CPT-II)**, which might reflect in utero toxicity of fatty acid metabolites or a developmental role for these enzymes. Progressive retinal degeneration, peripheral neuropathy, and chronic progressive liver disease have been identified in LCHAD and MTP deficiency.

Fatty acid oxidation disorders are easily overlooked because the only specific clue to the diagnosis may be the finding of inappropriately *low* concentrations of plasma or urinary ketones in an infant who has hypoglycemia unless specialized metabolic testing is performed. Genetic defects in ketone body utilization may also be overlooked because ketonemia is an expected finding with fasting hypoglycemia. Newborn screening programs using tandem mass spectrometry detect characteristic plasma acylcarnitine profiles in most of these disorders, allowing early and presymptomatic diagnosis. Screening programs have demonstrated that all the fatty acid oxidation disorders combined are among the most common inborn errors of metabolism, at least in predominantly White populations.

Figures 106.1 and 106.2 outline the steps involved in the oxidation of a typical long chain fatty acid. In the *carnitine cycle,* long chain fatty acids are transported across the barrier of the inner mitochondrial membrane as acylcarnitine esters. Medium-chain fatty acids, which are commonly provided as medium-chain triglyceride supplementation in infants who are failing to thrive, can bypass the carnitine cycle and enter the mitochondrial β-oxidation cycle directly. Within the mitochondria, successive rounds of the four-step *β-oxidation cycle* convert the **coenzyme A (CoA)**–activated fatty acids to acetyl-CoA units. Two or three different chain-length-specific isoenzymes are needed for each of these β-oxidation steps to accommodate the different chain-length fatty acyl-CoA species. The electrons generated in the first β-oxidation step (acyl-CoA dehydrogenase) are carried by the *electron transfer pathway* to the *electron transport chain* at the level of coenzyme Q for adenosine triphosphate production, whereas electrons generated from the third step (3-hydroxyacyl-CoA dehydrogenase) enter the *electron transport chain* at the level of complex 1. Most of the acetyl-CoA generated from fatty acid β-oxidation in the liver flows through the *pathway of ketogenesis* to form 3-hydroxybutyrate and acetoacetate, whereas in muscle and heart, the fatty acids are completely oxidized to CO_2 and water through oxidative phosphorylation. It has been demonstrated that the enzymes of fatty acid oxidation physically and functionally interact with each other and those of the mitochondrial electron transport chain.

DEFECTS IN THE β-OXIDATION CYCLE

Medium-Chain Acyl-CoA Dehydrogenase Deficiency

Medium-chain acyl-CoA dehydrogenase **(MCAD)** deficiency is the most common fatty acid oxidation disorder. The disorder shows a strong founder effect; most patients have a northwestern European ancestry, and the majority of these patients have at least one copy of a common MCAD missense pathogenic variant, an A-G transition at cDNA position 985 (c.985A>G) that changes a lysine to glutamic acid at residue 329 (p.Lys329Glu).

Clinical Manifestations

Previously undiagnosed affected patients usually present in the first 3 months to 5 years of life with episodes of acute illness triggered by prolonged fasting (>12-16 hours). Signs and symptoms include vomiting and lethargy, which rapidly progress to coma or seizures and cardiorespiratory collapse. Sudden unexpected infant death may occur. The liver may be slightly enlarged with fat deposition. Episodes are rare until the infant is beyond the first few months of life, presumably because of more frequent feedings at a younger age. Affected older infants are at higher risk of symptoms as they begin to fast through the night or are exposed to fasting stress during an intercurrent childhood illness. Presentation in the first days of life with neonatal hypoglycemia has been reported in newborns who were fasted inadvertently or were being breastfed exclusively and thus are at higher risk because of early reduced caloric intake. A diagnosis of MCAD has occasionally been documented in previously healthy teenage and adult individuals, indicating that even patients who have been asymptomatic in infancy are still at risk for metabolic decompensation if exposed to sufficient periods of fasting. An unknown number of patients may remain asymptomatic. Prior to routine newborn screening testing, as many as 25% of MCAD-deficient patients died or suffered severe brain injury from their first episode. Most patients are diagnosed in the newborn period by **blood spot acylcarnitine screening** (part of newborn screening), allowing the initiation of early intervention and prevention of most signs and symptoms.

Laboratory Findings

During acute episodes, hypoglycemia is usually present. Plasma and urinary ketone concentrations are inappropriately low **(hypoketotic**

Table 106.1 Mitochondrial Fatty Acid Oxidation Disorders: Clinical and Biochemical Features

ENZYME DEFICIENCY	GENE	CLINICAL PHENOTYPE	LABORATORY FINDINGS
Carnitine transporter	*SLC22A5*	Cardiomyopathy, skeletal myopathy, liver disease, sudden death, endocardial fibroelastosis, cardiac arrhythmia	↓ Total and free carnitine; normal acylcarnitines, acylglycine, and organic acids; prenatal and newborn possible diagnosis reported
Long chain fatty acid transporter	*FATP1-6*	Rare, acute liver failure in childhood requiring liver transplantation	↓ Intracellular C_{14}-C_{18} fatty acids; ↓ fatty acid oxidation; disease-causing pathogenic variants have not been identified in patients
Carnitine palmitoyl transferase-IA	*CPTIA*	Liver failure, renal tubulopathy, and sudden death reported; maternal preeclampsia; HELLP syndrome association described in a few patients	Normal or ↑ free carnitine; normal acylcarnitines, acylglycine, and organic acids; newborn screening diagnosis reported
Carnitine acylcarnitine translocase	*SLC25A20*	Chronic progressive liver failure, persistent ↑ NH_3, hypertrophic cardiomyopathy	Normal or ↓ free carnitine, abnormal acylcarnitine profile; newborn screening diagnosis reported
Carnitine palmitoyl transferase-II	*CPT2*	Early and late onset types; liver failure, encephalopathy, skeletal myopathy, cardiomyopathy, renal cystic changes; adult form with acute rhabdomyolysis, myoglobinuria	Normal or ↓ free carnitine; abnormal acylcarnitine profile; newborn screening diagnosis possible
Short chain acyl-CoA dehydrogenase	*ACADS*	Biochemical phenotype only; no consistent clinical phenotype	Normal or ↓ free carnitine; elevated urine ethylmalonic acid; inconsistently abnormal acylcarnitine profile
Medium-chain acyl-CoA dehydrogenase	*ACADM*	Hypoglycemia, hepatic encephalopathy, sudden death; maternal preeclampsia, HELLP syndrome association described rarely, possible long QT interval	Normal or ↓ free carnitine, ↑ urine acylglycine, plasma C_6-C_{10} free fatty acids, ↑ C_8-C_{10} acyl-carnitine; newborn screening diagnosis possible
Very long chain acyl-CoA dehydrogenase (VLCAD)	*ACADVL*	Dilated cardiomyopathy, arrhythmias, hypoglycemia, and hepatic steatosis; late-onset, stress-induced rhabdomyolysis, episodic myopathy	Normal or ↓ free carnitine; ↑ plasma $C_{14:1}$, C_{14} acylcarnitine; ↑ plasma C_{10}-C_{16} free fatty acids; prenatal and newborn screening diagnosis possible
ETF dehydrogenase*	*ETFDH*	Nonketotic fasting hypoglycemia, congenital anomalies, milder forms of liver disease, cardiomyopathy, and skeletal myopathy	Normal or ↓ free carnitine; increased ratio of acyl:free carnitine, ↑ blood acylcarnitines; characteristic urine organic acids and acylglycines; newborn screening diagnosis possible
ETF-α*	*ETFA*	Nonketotic fasting hypoglycemia, congenital anomalies, liver disease, cardiomyopathy; skeletal myopathy also described	Normal or ↓ free carnitine; increased ratio of acyl:free carnitine; ↑ acylcarnitines; urine organic acid and acylglycines; newborn screening diagnosis possible
ETF-β*	*ETFB*	Fasting hypoglycemia, congenital anomalies, liver disease, cardiomyopathy; skeletal myopathy also described	Normal or ↓ free carnitine; increased ratio of acyl:free carnitine, ↑ blood acylcarnitines; urine organic acid and acylglycines; newborn screening diagnosis possible
Short chain L-3-hydroxyacyl-CoA dehydrogenase (SCHAD)	*HAD1*	Hyperinsulinemic hypoglycemia, cardiomyopathy, myopathy	Normal or ↓ free carnitine, elevated free fatty acids, inconsistently abnormal urine organic acid, ↑ 3-OH glutarate, ↑ plasma C_4-OH acylcarnitine; newborn screening diagnosis possible
Long chain L-3-hydroxyacyl-CoA dehydrogenase (LCHAD)	*HADHA*	Maternal preeclampsia, HELLP syndrome, and AFLP association described frequently; see MTP later for clinical manifestations	Normal or ↓ free carnitine, increased ratio of acyl:free carnitine, ↑ free fatty acids, ↑ C_{16}-OH and C_{18}-OH carnitines; newborn screening diagnosis possible
MTP	*HADHA, HADHB*	Severe cardiac and skeletal myopathy, hypoglycemia, acidosis, hyper NH_3, sudden death, elevated liver enzymes, retinopathy; maternal preeclampsia, HELLP syndrome, and AFLP association described frequently	Normal or ↓ free carnitine, increased ratio of acyl:free carnitine, ↑ free fatty acids, ↑ C_{16}-OH and C_{18}-OH carnitines; newborn screening diagnosis possible
Long chain 3-ketoacyl-CoA thiolase	*HADHB*	Severe neonatal presentation, hypoglycemia, acidosis, ↑ creatine kinase, cardiomyopathy, neuropathy, and early death	Normal or ↓ free carnitine, increased ratio of acyl:free carnitine, ↑ free fatty acids, ↑ 2-*trans*, 4-*cis*-decadienoylcarnitine; newborn screening diagnosis possible
Short chain 2,3-enoyl-CoA hydratase	*ECHS1*	Leigh disease, lactic acidosis, seizures, cystic degeneration of white matter, microcephaly, metabolic acidosis, extrapyramidal dystonia, dilated cardiomyopathy	Abnormal organic acids, 2-methacrylglycine, 2-methyl-2,3 dihydroxybutyrate, also S-(2-carboxypropyl)cysteine, S-(2-carboxyethyl) cysteamine; acylcarnitine shows ↑ C4OH (inconsistently)

Table 106.1 Mitochondrial Fatty Acid Oxidation Disorders: Clinical and Biochemical Features—cont'd

ENZYME DEFICIENCY	GENE	CLINICAL PHENOTYPE	LABORATORY FINDINGS
2,4-Dienoyl-CoA reductase	*DECR1*	Only one patient described, hypotonia in the newborn, mainly severe skeletal myopathy and respiratory failure; hypoglycemia is rare	Normal or ↓ free carnitine, ↑ acyl:free carnitine ratio, normal urine organic acids and acylglycines
HMG CoA synthetase	*HMGCS2*	Hypoketosis and hypoglycemia, rarely myopathy	↑ Total plasma fatty acids; enzyme studies in biopsied liver may be diagnostic; genetic testing preferred
HMG CoA lyase	*HMGCL*	Hypoketosis and hypoglycemia, rarely myopathy	Normal free carnitine, ↑ C_5-OH, and methylglutaryl-carnitine; enzymes studies in fibroblasts may be diagnostic
Monocarboxylate transporter 1 (MCT1)	*SLC16A1*	Severe fasting-induced ketoacidosis, rarely hypoglycemia	Profound ketoacidosis; no specific biomarkers yet identified

*Also known as glutaric acidemia type II or multiple acyl-CoA dehydrogenase defect (MADD).
AFLP, Acute fatty liver of pregnancy; CoA, coenzyme A; ETF, electron transport flavoprotein; HELLP, hemolysis, elevated liver enzymes, low platelets; MTP, mitochondrial trifunctional protein; NH_3, ammonia.
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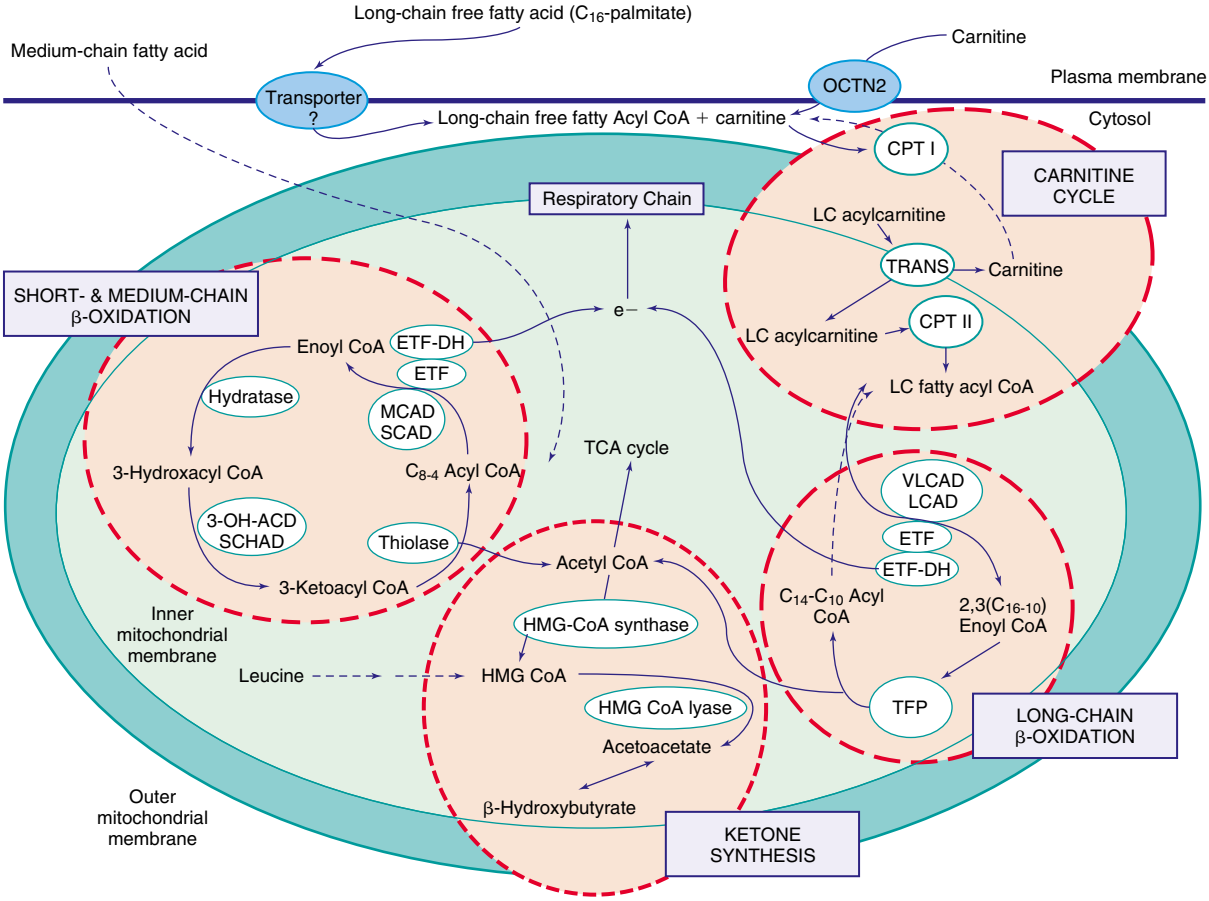

Fig. 106.1 Mitochondrial fatty acid oxidation. Carnitine enters the cell through the action of the organic cation/carnitine transporter (OCTN2). Palmitate, a typical 16-carbon long chain (LC) fatty acid, is transported across the plasma membrane and can be activated to form an LC fatty acyl coenzyme A (CoA). It then enters into the carnitine cycle, where it is transesterified by carnitine palmitoyltransferase-I (CPT-I), translocated across the inner mitochondrial membrane by carnitine/acylcarnitine translocase (TRANS), and then reconverted into an LC fatty acyl-CoA by carnitine palmitoyltransferase-II (CPT-II) to undergo β-oxidation. Very LC acyl-CoA dehydrogenase (VLCAD/LCAD) leads to the production of (C_{16}-C_{10}) 2,3-enoyl CoA. Mitochondrial trifunctional protein (MTP) contains the activities of enoyl CoA hydratase (hydratase), 3-OH-hydroxyacyl-CoA dehydrogenase (3-OH-ACD), and β-ketothiolase (thiolase). Acetyl-CoA, a reduced form of flavin adenine dinucleotide (FADH), and a reduced form of nicotinamide adenine dinucleotide (NADH) are produced. Medium- and short chain fatty acids (C8-4) can enter the mitochondrial matrix independent of the carnitine cycle. Medium-chain acyl-CoA dehydrogenase (MCAD), short chain acyl-CoA dehydrogenase (SCAD), and short chain hydroxy acyl-CoA dehydrogenase (SCHAD) are required. Acetyl-CoA can then enter the Krebs (TCA) cycle. Electrons are transported from FADH to the respiratory chain via the electron transfer flavoprotein (ETF) and the electron transfer flavoprotein dehydrogenase (ETF-DH). NADH enters the electron transport chain through complex I. In the liver, acetyl-CoA can be converted into hydroxymethylglutaryl (HMG) CoA by β-hydroxy-β-methylglutaryl-CoA synthase (HMG CoA synthase) and then the ketone body acetoacetate by the action of β-hydroxy-β-methylglutaryl-CoA lyase (HMG-CoA lyase).

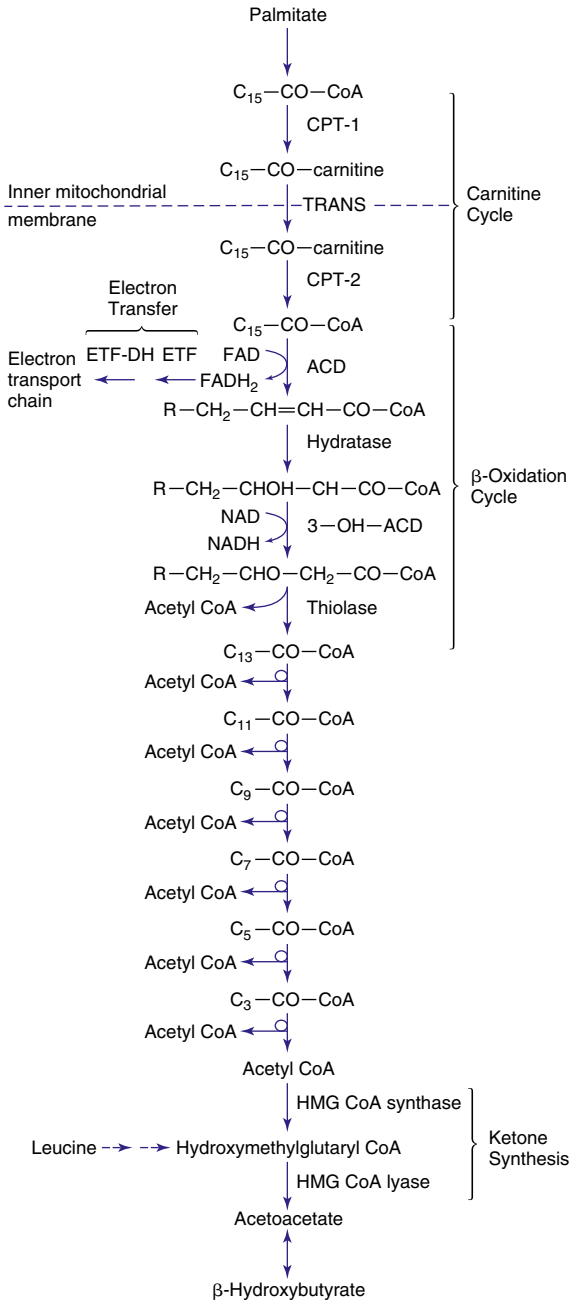

Fig. 106.2 Pathway of mitochondrial oxidation of palmitate, a typical 16-carbon long chain fatty acid. Enzyme steps include carnitine palmitoyltransferase (CPT) 1 and 2, carnitine/acylcarnitine translocase (TRANS), electron transfer flavoprotein (ETF), ETF dehydrogenase (ETF-DH), acyl-CoA dehydrogenase (ACD), enoyl CoA hydratase (hydratase), 3-hydroxy-acyl-CoA dehydrogenase (3-OH-ACD), β-ketothiolase (thiolase), β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) synthase, and lyase.

hypoglycemia). Because of the hypoketonemia, there is little or no metabolic acidosis, which is typically present in children with hypoglycemia not caused by fatty acid oxidation disorders. Liver function tests (LFTs) are abnormal, with elevations of liver enzymes (alanine transaminase, aspartate transaminase), elevated blood ammonia, and prolonged prothrombin and partial thromboplastin times. Liver biopsy at times of acute illness shows microvesicular or macrovesicular steatosis from triglyceride accumulation. During fasting stress or acute illness, urinary organic acid profiles by gas chromatography/mass spectrometry show inappropriately low concentrations of ketones and elevated levels of medium-chain dicarboxylic acids (adipic, suberic, and sebacic acids) that derive from microsomal and peroxisomal omega oxidation of accumulated medium-chain fatty acids. Plasma and tissue concentrations of total carnitine are reduced to 25–50% of normal, and the fraction of total esterified carnitine is increased. This pattern of **secondary carnitine deficiency** is seen in most fatty acid oxidation defects and reflects competition between increased acylcarnitine levels and free carnitine for transport at the renal tubular plasma membrane. Significant exceptions to this rule are the plasma membrane carnitine transporter, CPT-IA, and β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) synthase deficiencies, which do not manifest secondary carnitine deficiency.

Diagnostic metabolite patterns for MCAD deficiency include increased plasma $C_{6:0}$, $C_{8:0}$, $C_{10:0}$, and $C_{10:1}$ acylcarnitine species and increased urinary acylglycines, including hexanoylglycine, suberylglycine, and 3-phenylpropionylglycine. Newborn screening, performed in all states in the United States and in many other countries, can detect presymptomatic MCAD deficiency based on these abnormal acylcarnitines in filter paper blood spots. The diagnosis can be confirmed by demonstrating the common Ala985Gly pathogenic variant or sequencing the MCAD gene. A second common variant, Thr199Cys, has been detected in infants identified by newborn screening. Interestingly, this allele has not been seen to date in symptomatic MCAD patients, likely because of the significant residual activity of this milder pathogenic variant.

Treatment

Acute illnesses should be promptly treated with intravenous (IV) fluids containing 10% dextrose to correct or prevent hypoglycemia and to suppress lipolysis as rapidly as possible (see Chapter 113). Chronic therapy consists of avoiding fasting. This usually requires simply adjusting the diet to ensure that overnight fasting periods are limited to <6 hours in infants under 6 months of age, <8 hours for infants 6-24 months of age, and <12 hours for older children. Restricting dietary fat or treatment with carnitine is unnecessary. There is likely no need for active therapeutic intervention for individuals with the Thr199Cys variant.

Prognosis

Up to 25% of previously undiagnosed patients may die during their first attack of illness. There is often a history of a previous sibling death that is presumed to be from an unrecognized MCAD deficiency. Some patients may sustain permanent brain injury during an attack of profound hypoglycemia. For survivors without brain damage and essentially all babies identified by newborn screening, the prognosis is excellent because progressive cognitive impairment or cardiomyopathy does not occur in MCAD deficiency. Fasting tolerance improves with age, and the risk of decompensation decreases. Because as many as 35% of affected patients have never had an episode, testing of siblings of affected patients is important to detect asymptomatic family members who did not receive newborn screening.

Very Long Chain Acyl-CoA Dehydrogenase Deficiency

Very long chain acyl-CoA dehydrogenase **(VLCAD)** deficiency is the second most common diagnosed disorder of fatty acid oxidation. It was originally termed "long chain acyl-CoA dehydrogenase deficiency" before the existence of the inner mitochondrial membrane-bound VLCAD was known. *All patients previously diagnosed as having long chain acyl-CoA dehydrogenase deficiency have* VLCAD *gene defects.* Patients with VLCAD deficiency have no ability to oxidize physiologic long chain fatty acids and are usually more severely affected than those with MCAD deficiency, who have a milder oxidative defect. VLCAD deficiency presents earlier in infancy and has more chronic problems with muscle weakness or episodes of muscle pain and rhabdomyolysis. Cardiomyopathy may be present during acute attacks provoked by fasting. The left ventricle may be hypertrophic or dilated and may show poor contractility on echocardiography. Sudden unexpected death has occurred in several patients, but most who survive the initial episode show improvement, including normalization of cardiac function.

Other physical and routine laboratory features are similar to those of MCAD deficiency, including secondary carnitine deficiency. The urinary organic acid profile shows a nonketotic dicarboxylic aciduria with increased levels of $C_{6\text{-}12}$ dicarboxylic acids. Diagnosis may be suggested by an abnormal acylcarnitine profile with plasma or blood spot $C_{14:0,\ 14:1,\ 14:2}$ acylcarnitine species. However, the specific diagnosis requires pathogenic variant analysis of the *VLCAD* gene. Newborn screening by tandem mass spectrometry of dried blood spots is effective in identifying early/severe disease, though it can miss milder, later-onset cases. A common mild variant is identified by a newborn screen that typically predicts later-onset disease. Treatment has traditionally been based primarily on avoidance of fasting for >8-12 hours depending on age, along with supplementation with medium-chain triglycerides. Triheptanoin (Dojolvi) has been approved as a treatment for VLCAD deficiency and for all other disorders of long-chain fatty acid oxidation. The odd-chain fat heptanoate (C7) released from this triglycerol provides acetyl-CoA for the tricarboxylic acid cycle *and* propionyl-CoA, which is metabolized through methylmalonyl-CoA to succinyl-CoA and succinate. This anaplerotic effect essentially eliminates hypoglycemia in long-chain fatty acid oxidation disorder patients and significantly improves heart function in patients with cardiomyopathy. However, although heptanoin does reduce the incidence, rhabdomyolysis remains a major problem in treated patients. This finding led to the recognition of previously unrecognized inflammation as a component of these disorders, likely caused by the accumulation of a high level of proinflammatory long-chain complex lipids. Patients can develop a secondary carnitine deficiency; however, the need for replacement therapy remains unclear. If the free carnitine level in blood is <10 μM, supplementation with 25-50 mg/kg of oral carnitine (with a maximum of 1,000 mg/day) may be helpful, especially in the face of cardiomyopathy or significant muscular symptoms.

Short Chain Acyl-CoA Dehydrogenase Deficiency

A small number of patients with two null pathogenic variants in the short chain acyl-CoA dehydrogenase (SCAD) gene have been described with a variable phenotype. Most individuals classified as being SCAD deficient actually have polymorphic DNA changes in the *SCAD* gene; two common polymorphisms are Gly185Ser and Arg-147Trp, present in biallelic fashion in 7% of the population. Although SCAD deficiency was originally reported with a wide range of symptoms, it was likely a result of the frequency of the common polymorphisms in unrelated disease rather than a true causal relationship. Long-term follow-up of infants identified by newborn screening has failed to demonstrate a convincing, consistent phenotype, and thus SCAD deficiency is best described as a biochemical phenotype rather than a disease. The diagnosis is indicated by elevated levels of butyrylcarnitine (C4-carnitine) on newborn blood spots or plasma and increased excretion of urinary ethylmalonic acid and butyrylglycine. These metabolic abnormalities are most pronounced in patients with null pathogenic variants and are variably present in patients who are homozygous for the common polymorphisms. In the context of metabolic abnormalities consistent with SCAD deficiency in patients with significant clinical problems, a thorough evaluation for *another* unrelated diagnosis is indicated. There is no need for treatment of individuals with SCAD deficiency.

Long Chain 3-Hydroxyacyl-CoA Dehydrogenase/ Mitochondrial Trifunctional Protein Deficiency

The LCHAD enzyme is part of the MTP, which also contains two other steps in β-oxidation: long chain 2,3-enoyl CoA hydratase and long chain β-ketothiolase. MTP is a heterotetrameric protein composed of two α and two β chains derived from distinct contiguous genes, *HADHA* and *HADHB,* that share a common promoter. In some patients, only the LCHAD activity of the MTP is affected **(LCHAD deficiency)**, whereas others have deficiencies of all three activities **(MTP deficiency)**.

Clinical manifestations include attacks of acute hypoketotic hypoglycemia similar to VLCAD deficiency; however, patients often show evidence of more severe disease, including cardiomyopathy, muscle cramps and weakness, and abnormal liver function (cholestasis). Pigmented retinopathy leading to blindness, progressive liver failure, peripheral neuropathy, and rhabdomyolysis are also present, with retinopathy being more severe in isolated LCHAD deficiency and neuropathy worse in combined MTP defects. Life-threatening obstetric complications (AFLP, HELLP syndrome) have been observed in heterozygous mothers carrying homozygous fetuses affected with LCHAD/MTP deficiency. Sudden unexpected infant death may occur, especially in populations where tandem mass spectrometry newborn screening is not routine. The **diagnosis** is indicated by elevated levels of blood spot or plasma 3-hydroxy acylcarnitines of chain lengths C_{16}-C_{18}. The urinary organic acid profile in patients may show increased levels of 3-hydroxydicarboxylic acids of chain lengths C_6-C_{14}. Secondary carnitine deficiency is common. A common pathogenic variant in the α subunit, E474Q, is seen in more than 60% of isolated LCHAD-deficient patients. This pathogenic variant in the fetus is especially associated with the obstetric complications, but other pathogenic variants in either subunit may also be linked to maternal illness.

Treatment is similar to that for VLCAD deficiency; that is, avoiding fasting stress, triheptanoin, and potentially carnitine. Docosahexaenoic acid may slow the retinal changes but does not prevent them. None of these therapeutic measures likely affect the development or progression of peripheral neuropathy. Liver transplantation has been attempted in patients with severe liver failure but does not ameliorate the metabolic abnormalities or prevent the myopathic or retinal complications.

Short Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency

Very few patients with proven pathogenic variants of short chain 3-hydroxyacyl-CoA dehydrogenase **(SCHAD)** have been reported. Most cases with recessive pathogenic variants of the SCHAD gene have presented with episodes of hypoketotic hypoglycemia that was caused by *hyperinsulinism*. In contrast to those with other forms of fatty acid oxidation disorders, these patients required specific therapy with diazoxide for hyperinsulinism to avoid recurrent hypoglycemia. A single patient with compound heterozygous pathogenic variants presented with fulminant hepatic failure at age 10 months. The SCHAD protein has a nonenzymatic function in which it directly interacts with glutamate dehydrogenase (GDH) to inhibit its activity. In the absence of SCHAD protein, this inhibition is removed, leading to upregulation of GDH enzyme activity, a recognized cause of *hyperinsulinism,* usually from activating pathogenic variants of the *GDH* gene. Severe deficiency of SCHAD protein often presents predominantly as **protein-sensitive hypoglycemia** rather than as fasting hypoglycemia. It appears that if SCHAD protein is present, inhibition of GDH is maintained even when there is no SCHAD enzyme activity; these patients may present with a more traditional fatty acid oxidation defect. Specific metabolic markers for SCHAD deficiency include elevated plasma C4-hydroxy acylcarnitine and urine 3-hydroxyglutaric acid. Successful newborn screening for SCHAD deficiency has been recorded, but the sensitivity of the process has not yet been established.

Treatment of SCHAD-deficient patients with hyperinsulinism is with diazoxide. There is insufficient experience with the non-hyperinsulinemic form of SCHAD deficiency at present to recommend treatment modalities, but prevention of fasting seems advisable.

Short Chain 2,3-Enoyl-CoA Hydratase Deficiency

This rare disorder, resulting from pathogenic variants in the *ECHS1* gene, was identified through exome sequencing. The disorder affects a shared pathway of short chain fatty acid and valine metabolism. The clinical phenotypes are more characteristic of mitochondrial disorders of pyruvate metabolism with predominantly a **Leigh-like disease** (see Chapters 108 and 638.2) with profound and often-fatal lactic acidosis. No treatment modalities or specific biomarkers have been established. Several patients were found to excrete increased levels of methacrylylglycine, a highly reactive and potentially toxic intermediate; 2-methyl-2.3 dihydroxybutyrate; *S*-(2-carboxypropyl) cysteine; and *S*-(2-carboxpropyl) cysteamine.

DEFECTS IN THE CARNITINE CYCLE

Plasma Membrane Carnitine Transport Defect (Primary Carnitine Deficiency)

Primary carnitine deficiency is the only genetic defect in which carnitine deficiency is the *cause,* rather than the consequence, of impaired fatty acid oxidation. The most common presentation is progressive **cardiomyopathy** with or without **skeletal muscle weakness** beginning at age 1-4 years. A smaller number of patients may present with fasting hypoketotic hypoglycemia in the first year of life, before the cardiomyopathy becomes evident. Cardiac arrythmias are often seen. A common pathogenic variant causes late-onset disease in the Faroe Islands with sudden death caused by cardiomyopathy and/or arrhythmia. The underlying defect involves the high-affinity plasma membrane sodium gradient–dependent carnitine transporter encoded by the *SLC22A5* gene that is expressed at high levels in heart, muscle, and kidney. This transporter is responsible both for maintaining intracellular carnitine concentrations 20- to 50-fold higher than plasma concentrations and for renal conservation of carnitine.

Diagnosis of the carnitine transporter defect is aided by patients having extremely reduced carnitine levels in plasma and muscle (1–2% of normal). Heterozygote parents have plasma carnitine levels approximately 50% of normal. Fasting ketogenesis may be normal because liver carnitine transport is normal, but it may become impaired if dietary carnitine intake is interrupted. The fasting urinary organic acid profile may show a hypoketotic dicarboxylic aciduria pattern if hepatic fatty acid oxidation is impaired but is otherwise unremarkable. The defect in carnitine transport can be demonstrated clinically by the severe reduction in renal carnitine threshold or by in vitro assay of carnitine uptake using cultured fibroblasts or lymphoblasts. *SLC22A5* sequencing is the most common method to confirm the diagnosis. A common relatively severe pathogenic variant has been described in the Chinese population in Taiwan. **Treatment** with pharmacologic doses of oral carnitine (100-300 mg/kg/day) is highly effective in correcting the cardiomyopathy and muscle weakness, as well as any impairment in fasting ketogenesis. Muscle total carnitine concentrations remain <5% of normal on treatment.

Carnitine Palmitoyltransferase-IA Deficiency

Several dozen infants and children have been described with a deficiency of the liver and kidney CPT-I isozyme (CPT-IA). **Clinical manifestations** include fasting-induced hypoketotic hypoglycemia, occasionally with extremely abnormal LFTs and, rarely, with renal tubular acidosis. The heart and skeletal muscles are not involved because the muscle isozyme is unaffected. Fasting urinary organic acid profiles sometimes show a hypoketotic C_6-C_{12} dicarboxylic aciduria but may be normal. Plasma acylcarnitine analysis demonstrates mostly free carnitine with very little acylated carnitine. This observation has been used to identify CPT-IA deficiency on newborn screening by tandem mass spectrometry. CPT-IA deficiency is the only fatty acid oxidation disorder in which plasma total carnitine levels may be elevated, often to 150–200% of normal. This phenomenon is explained by the absence of inhibitory effects of long chain acylcarnitines on the renal tubular carnitine transporter in CPT-IA deficiency. The enzyme defect can be demonstrated in cultured fibroblasts or lymphoblasts. CPT-IA deficiency in the fetus has been associated in a single case report with AFLP in the mother. A common variant in the *CPTIA* gene (c.1436C>t, p.Pro479Leu) has been identified in individuals of Inuit background in the United States, Canada, and Greenland. This variant is associated with an increased risk for sudden infant death syndrome (SIDS) in the Inuit population. The variant can be detected by newborn screening; enzyme activity is reduced by 80%, and regulation by malonyl-CoA is lost. It has not been established whether this variant is a pathologic enzyme variant or an adaptation to ancient Inuit high-fat diets. Another pathogenic variant is common in the Ashkenazi Jewish population and leads to infantile symptoms. **Treatment** for the severe form of CPT-IA deficiency that is found in non-Inuit populations is similar to that for VLCAD deficiency, with avoidance of situations where fasting ketogenesis is necessary and use of triheptanoin to provide an anaplerotic fuel source. The need for treatment of the Inuit variant has not yet been determined.

Carnitine:Acylcarnitine Translocase Deficiency

This defect of the inner mitochondrial membrane carrier protein for long chain acylcarnitines blocks the entry of long chain fatty acids into the mitochondria for oxidation. The clinical phenotype of this disorder is driven by a typically severe and generalized impairment of fatty acid oxidation. Most newborn patients present with attacks of fasting-induced hypoglycemia, hyperammonemia, and cardiorespiratory collapse. All symptomatic newborns have had evidence of cardiomyopathy and muscle weakness. Several patients with a partial translocase deficiency and milder disease without cardiac involvement have also been identified. No distinctive urinary or plasma organic acids are noted, although increased levels of plasma long chain acylcarnitines of chain lengths C_{16}-C_{18} are reported, not distinguishable from the pattern seen in CPT-II deficiency (see later). **Diagnosis** can be confirmed using genetic analysis. Functional carnitine:acylcarnitine translocase activity can be measured in cultured fibroblasts or lymphoblasts. **Treatment** is similar to that of VLCAD deficiency and is particularly effective in reducing the hyperammonemia that is often otherwise persistent in severe patients.

Carnitine Palmitoyltransferase-II Deficiency

Three forms of CPT-II deficiency have been described. A **severe neonatal lethal** presentation associated with a profound enzyme deficiency, and early death has been reported in newborns with hypoglycemia and hyperammonemia in association with dysplastic kidneys, cerebral malformations, and mild facial anomalies. A milder defect is associated with an **adult presentation** of episodic rhabdomyolysis. The first episode usually does not occur until late childhood or early adulthood. Attacks are frequently precipitated by prolonged exercise. There is aching muscle pain and myoglobinuria that may be severe enough to cause renal failure. Hypoglycemia has not been described, but fasting may contribute to attacks of myoglobinuria. Muscle biopsy shows increased deposition of neutral fat. This adult myopathic presentation of CPT-II deficiency is associated with a common *CPT2* pathogenic variant, c.338C>T, p.Ser113Leu, seen as a recurrent pathogenic variant in the Ashkenazi Jewish population. This pathogenic variant produces a heat-labile protein that is unstable to increased muscle temperature during exercise that may contribute to the myopathic presentation. A third **intermediate form** of CPT-II deficiency presents in infancy or early childhood with fasting-induced hepatic failure, cardiomyopathy, and skeletal myopathy with hypoketotic hypoglycemia but is not associated with the severe developmental changes seen in the neonatal lethal presentation. This pattern of illness is similar to VLCAD deficiency, and management is identical. It is often caused by the presence of one severe pathogenic variant in combination with the mild c.338C>T variant.

Diagnosis of all forms of CPT-II deficiency can be made by a combination of molecular genetic analysis and demonstrating deficient enzyme activity in muscle or other tissues and in cultured fibroblasts. Severe and intermediate disease can be identified through newborn screening, but patients homozygous for the common mild variant typically have normal newborn screens.

DEFECTS IN THE ELECTRON TRANSFER PATHWAY

Electron Transfer Flavoprotein and Electron Transfer Flavoprotein Dehydrogenase Deficiencies (Glutaric Acidemia Type 2, Multiple Acyl-CoA Dehydrogenation Defects)

Electron transfer flavoprotein (ETF) and electron transfer flavoprotein dehydrogenase (ETF-DH) function to transfer electrons into the mitochondrial electron transport chain from dehydrogenation reactions catalyzed by VLCAD, MCAD, SCAD, and glutaryl-CoA dehydrogenase, four enzymes involved in branched-chain amino acid (BCAA) oxidation and sarcosine and dimethylglycine dehydrogenases. Deficiencies of ETF or ETF-DH produce illness that combines the features of impaired fatty acid oxidation and impaired oxidation of several amino acids. Complete deficiencies of either protein are associated with severe illness in the newborn period, characterized by acidosis, hypoketotic hypoglycemia, coma, hypotonia, cardiomyopathy, and

an unusual odor of sweaty feet caused by isovaleryl-CoA dehydrogenase inhibition. Some affected neonates have had congenital facial dysmorphism and polycystic kidneys similar to those in severe CPT-II deficiency, which suggests that toxic effects of accumulated metabolites may occur in utero. Most severely affected infants do not survive the neonatal period. Disorders of cellular or mitochondrial riboflavin transport or flavin adenine dinucleotide (FAD, an essential cofactor for the acyl-CoA dehydrogenases) have been identified with similar biochemical profiles to ETF and ETF-DH deficiency but with more variable features.

Diagnosis can be made from the newborn blood spot acylcarnitine profile and urinary organic acids; both tests show abnormalities corresponding to disruptions in the oxidation of fatty acids (ethylmalonate and C_6-C_{10} dicarboxylic acids), lysine (glutarate), and BCAAs (isovaleryl-, isobutyryl-, and 2-methylbutyrylglycine). The diagnosis can be confirmed by genetic testing of *ETFA, ETFB,* and *ETFDH*. If no pathogenic variants in these genes are identified, additional testing for the riboflavin transport and FAD synthesis genes should be pursued.

Partial deficiencies of ETF and ETF-DH produce a disorder that may mimic MCAD deficiency or other milder long-chain fatty acid oxidation defects with attacks of fasting hypoketotic coma. The urinary organic acid profile reveals primarily elevations of dicarboxylic acids and ethylmalonate, derived from short chain fatty acid intermediates. Secondary carnitine deficiency is present. Some patients with mild forms of ETF/ETF-DH deficiency may benefit from treatment with high doses of *riboflavin,* a precursor of the various flavoproteins involved in electron transfer.

DEFECTS IN THE KETONE SYNTHESIS PATHWAY

The final steps in production of ketones from mitochondrial fatty acid β-oxidation convert acetyl-CoA to acetoacetate through two enzymes of the HMG-CoA pathway (see Fig. 106.2).

β-Hydroxy-β-Methylglutaryl-CoA (HMG-CoA) Synthase Deficiency

See Chapter 103.6.

HMG-CoA synthase is the rate-limiting step in the conversion of acetyl-CoA derived from fatty acid β-oxidation in the liver to ketones. The presentation of deficiency is one of fasting hypoketotic hypoglycemia without evidence of impaired cardiac or skeletal muscle function. The urinary organic acid profile shows only a nonspecific hypoketotic dicarboxylic aciduria. Plasma and tissue carnitine levels are normal, in contrast to all the other disorders of fatty acid oxidation. A separate synthase enzyme, present in cytosol for cholesterol biosynthesis, is not affected. The HMG-CoA synthase defect is expressed only in the liver and kidney and cannot be demonstrated in cultured fibroblasts. The **diagnosis** can be made by genetic testing. *Avoiding fasting is usually a successful treatment.*

β-Hydroxy-β-Methylglutaryl-CoA Lyase Deficiency (3 Hydroxy-3-Methylgutaric Aciduria)

See Chapter 105.6.

DEFECTS IN KETONE BODY UTILIZATION

The ketone bodies β-hydroxybutyrate and acetoacetate are the end products of hepatic fatty acid oxidation and are important metabolic fuels for the brain during fasting. Three defects in utilization of ketones in brain and other peripheral tissues present as episodes of **hyperketotic coma**, with or without hypoglycemia.

Monocarboxylate Transporter-1 Deficiency

About 10 patients have been described with recurrent episodes of potentially lethal ketoacidosis, with or without hypoglycemia, caused by a deficiency of monocarboxylate transporter 1 (MCT1), a plasma membrane carrier encoded by *SLC16A1* that is required to transport ketones into tissues from plasma. Although the first cases identified were homozygous for inactivating pathogenic variants of *SLC16A1,* heterozygous carriers can also be affected. Affected patients develop severe ketoacidosis provoked by fasting or infections in their first years of life; hypoglycemia is not always present. The differential includes ketotic hypoglycemia associated with milder forms of glycogen storage disease, such as phosphorylase or phosphorylase kinase deficiency (see Chapter 107). **Treatment** for acute episodes includes IV dextrose to suppress lipolysis and inhibit ongoing ketogenesis. Long-term treatment includes avoidance of prolonged fasting stress. The **diagnosis** should be suspected by unusually severe ketosis and delayed suppression of ketones after starting treatment with dextrose. There are no specific metabolic markers or newborn screening methods. The diagnosis can be established by genetic sequencing of *SLC16A1*.

Succinyl-CoA:3-Ketoacid-CoA Transferase Deficiency

See Chapter 103.6.

The characteristic presentation of succinyl-CoA:3-ketoacid-CoA transferase **(SCOT)** deficiency is an infant with recurrent episodes of severe ketoacidosis induced by fasting. Plasma acylcarnitine and urine organic acid abnormalities do not distinguish SCOT deficiency from other causes of ketoacidosis. **Treatment** of episodes requires infusion of glucose and large amounts of bicarbonate until metabolic stability is reestablished. Patients usually exhibit inappropriate, persistent hyperketonemia even between episodes of illness. SCOT is responsible for activating acetoacetate in peripheral tissues, using succinyl CoA as a donor to form acetoacetyl-CoA. Deficient enzyme activity can be demonstrated in the brain, muscle, and fibroblasts from affected patients. The gene has been cloned, and numerous pathogenic variants have been characterized. The diagnosis is usually established by sequencing analysis of the *OXCT1* gene that encodes the SCOT enzyme.

β-Ketothiolase Deficiency

See Chapter 105.6.

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106.2 Disorders of Very Long Chain Fatty Acids and Other Peroxisomal Functions

Michael F. Wangler

PEROXISOMAL DISORDERS

Disorders of very long chain fatty acids (VLCFAs) fall within the broader group of peroxisomal diseases. The peroxisomal diseases are genetically determined disorders caused either by the failure to form or maintain the peroxisome or by a defect in the function of a single protein that is localized to the peroxisome. These disorders cause serious disability in childhood and occur more frequently and present a wider range of phenotypes than recognized in the past. Many, but not all, peroxisomal disorders are associated with elevations of VLCFAs.

Etiology

Peroxisomal disorders are subdivided into two major categories (Table 106.2). In the **peroxisomal biogenesis disorders (PBDs)**, the basic defect is the failure to import one or more proteins into the organelle. In the other group, defects affect a single peroxisomal protein (**single-enzyme defects**). The *peroxisome* is present in all cells except mature erythrocytes and is a subcellular organelle surrounded by a single membrane; there are >50 peroxisomal enzymes. Some enzymes are involved in the production and decomposition of hydrogen peroxide and others in lipid and amino acid metabolism. Peroxisomal enzymes have a unique system to ship them to the peroxisome that uses specific **peroxisome targeting sequences** (PTSs). These enzymes are first synthesized in their mature form on free polyribosomes and float free in the cytoplasm until their specific PTS is recognized. Most peroxisomal matrix proteins contain a **PTS1**, a short amino acid sequence at the carboxyl terminus. In addition, the amino-terminal–located **PTS2** is critical for the import of enzymes involved in plasmalogen

Table 106.2 Peroxisomal Disorder Classification, Disorders, and Genes

PEROXISOMAL BIOGENESIS DISORDERS	GENES
Peroxisome biogenesis disorder–Zellweger spectrum disorders (PBD-ZSD) Zellweger syndrome (severe PBD-ZSD) Neonatal adrenoleukodystrophy (intermediate PBD-ZSD) Infantile Refsum disease (mild PBD-ZSD)	*PEX1, PEX2, PEX3, PEX5, PEX6, PEX10, PEX11B, PEX12, PEX13, PEX14, PEX16, PEX19, PEX26*
Rhizomelic chondrodysplasia punctata, types 1 and 5 (RCDP1 and RCDP5)	*PEX7* and *PEX5*, respectively
SINGLE-ENZYME DEFECTS	
X-linked adrenoleukodystrophy	*ABCD1*
Acyl-CoA oxidase deficiency	*ACOX1*
D-Bifunctional protein deficiency	*HSD17B4*
2-Methylacyl-CoA racemase deficiency	*AMACR*
Rhizomelic chondrodysplasia punctata, types 2 and 3 (RCDP2 and RCDP3)	*AGPS, GNPAT*
Adult Refsum disease	*PHYH*

Table 106.3 Abnormal Laboratory Findings Common to Zellweger Spectrum Disorders

Defective oxidation and abnormal accumulation of very long chain fatty acids
Peroxisomes absent to reduced in number
Catalase in cytosol
Deficient synthesis and reduced tissue levels of plasmalogens
Deficient oxidation and age-dependent accumulation of phytanic acid
Defects in certain steps of bile acid formation and accumulation of bile acid intermediates
Defects in oxidation and accumulation of L-pipecolic acid
Increased urinary excretion of dicarboxylic acids

and branched-chain fatty acid metabolism. The peroxisome biogenesis machinery then recognizes, binds, and targets PTS-containing proteins to the peroxisome. This process involves a complex series of reactions mediated by at least 23 distinct proteins. These proteins, referred to as *peroxins,* are encoded by the *PEX* genes. Pathogenic genetic variants in these genes are the cause of PBDs.

Epidemiology

Except for X-linked adrenoleukodystrophy (ALD), all the peroxisomal disorders listed in Table 106.2 are inherited as *autosomal recessive diseases*. ALD is the most common peroxisomal disorder, with an estimated incidence of 1 in 17,000 live births. The combined incidence of the other peroxisomal disorders is estimated to be 1 in 50,000 live births, although with broader newborn screening, it is expected that the actual incidences of all the disorders of VLCFAs will be more accurately established.

Pathology

Absence or reduction in the number of peroxisomes is pathognomonic for disorders of peroxisome biogenesis. In most cases, a close examination of cells in these patients reveals membranous sacs that lack the normal complement of matrix proteins; these are peroxisome "ghosts," and they indicate the inability of the cell to properly localize peroxisomal proteins. Pathologic changes are observed in most organs and include profound and characteristic defects in neuronal migration, micronodular cirrhosis of the liver, renal cysts, chondrodysplasia punctata, sensorineural hearing loss, retinopathy, congenital heart disease, and dysmorphic features.

Pathogenesis

Clinical pathologic changes of the PBDs are secondary to the underlying peroxisome biogenesis defect. As a result, multiple peroxisomal enzymes fail to function in the PBDs (Table 106.3). PBDs include the Zellweger spectrum disorders **(PBD-ZSD)** and one form of rhizomelic chondrodysplasia punctata **(PBD-RCDP)**, which are distinguished by clinical phenotype and differences in the extent of biogenesis abnormality. In PBDs, enzymes that are synthesized cannot be properly located to the peroxisome and are thus degraded abnormally quickly because they are unprotected outside the peroxisome. However, it has yet to be clarified as to how specific peroxisome defects lead to each of the pathologic manifestations.

Pathogenic variants in 14 different *PEX* genes have been identified in PBDs. For PBD-RCDP, *PEX7* pathogenic variants are the most common, but rarer *PEX5* pathogenic variants are seen. The pattern and severity of pathologic features vary with the nature of the import defects and the degree of import impairment leading to the severity spectrum of PBD-ZSD. These gene defects lead to disorders named prior to recognizing their relationship to the peroxisome, namely, Zellweger syndrome, neonatal ALD, infantile Refsum disease, and RCDP. The first three disorders are considered to be a form of *clinical continuum,* with Zellweger syndrome the most severe **(severe PBD-ZSD)**, infantile Refsum disease the least severe **(mild PBD-ZSD)**, and neonatal ALD being intermediate **(intermediate PBD-ZSD)**. For PBD-ZSD, 13 genes can be affected to result in autosomal recessive disease *(PEX1, PEX2, PEX3, PEX5, PEX6, PEX10, PEX11B, PEX12, PEX13, PEX14, PEX16, PEX19, PEX26)*. Specific gene defects cannot be distinguished by clinical features. Clinical severity varies with the degree to which protein import is impaired. Pathogenic variants that completely abolish import are often associated with the severe PBD-ZSD or Zellweger phenotype, whereas a missense variant, in which some degree of import function is retained, leads to the somewhat milder presentations. A defect in *PEX7* or, very rarely, *PEX5*, which depend on peroxisomal import that use PTS2, is associated with RCDP. *PEX7* defects that leave the import partially intact are associated with milder phenotypes, some of which resemble classic (adult) Refsum disease.

The genetic disorders that involve single peroxisomal enzymes usually have clinical manifestations that are more restricted and relate to the single biochemical defect. The primary adrenal insufficiency of ALD is caused by an accumulation of VLCFAs in the adrenal cortex, and the peripheral neuropathy in adult Refsum disease is caused by the accumulation of phytanic acid in Schwann cells and myelin.

PBD-ZSD

Newborn infants with *severe* PBD-ZSD, previously described as **Zellweger syndrome**, show striking and consistent recognizable abnormalities. Of central diagnostic importance are the typical facial appearance (large anterior fontanelle, wide sutures, high forehead, hypoplastic supraorbital ridges, flat face, and broad nasal bridge; Fig. 106.3), severe weakness and hypotonia, neonatal seizures, and eye abnormalities. Because of the hypotonia and craniofacial appearance, Down syndrome may be suspected in neonates. Infants with severe PBD-ZSD rarely live more than a few months. More than 90% show postnatal growth failure. Table 106.4 lists the main clinical abnormalities.

Patients with *intermediate* PBD-ZSD, previously described as **neonatal ALD**, show fewer, less prominent craniofacial features. Neonatal seizures occur frequently. Psychomotor developmental delay is present; function remains in the range of severe intellectual disability, and development may regress after 3-5 years of age, likely from progressive leukodystrophy. Hepatomegaly, impaired liver function, pigmentary degeneration of the retina, and severely impaired hearing are invariably present. Adrenocortical function is usually impaired and may require adrenal hormone replacement. Chondrodysplasia punctata and renal cysts are absent.

Patients with *mild* PBD-ZSD, previously described as **infantile Refsum disease**, have survived to adulthood. They can walk, although gait may be ataxic and broad based. Cognitive function is generally

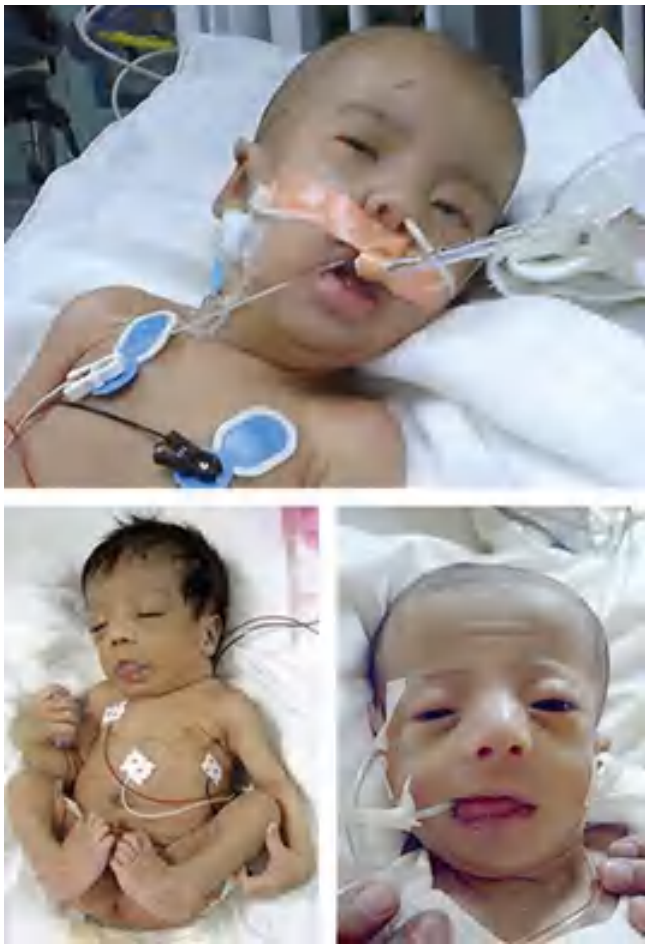

Fig. 106.3 Zellweger syndrome. Three affected neonates. Note the hypotonia, high forehead with shallow supraorbital ridges, anteverted nares, and mild micrognathia, as well as the talipes equinovarus and contractures at the knees. *(From Shaheen R, Al-Dirbashi OY, Al-Hassnan ZN, et al. Clinical, biochemical and molecular characterization of peroxisomal diseases in Arabs. Clin Genet. 2011;79[1]:60–70.)*

Table 106.4 Main Clinical Abnormalities in Zellweger Syndrome

	PATIENTS IN WHOM THE FEATURE WAS PRESENT	
ABNORMAL FEATURE	**NUMBER**	**%**
High forehead	58	97
Flat occiput	13	81
Large fontanelle(s), wide sutures	55	96
Shallow orbital ridges	33	100
Low/broad nasal bridge	23	100
Epicanthus	33	92
High-arched palate	35	95
External ear deformity	39	97
Micrognathia	18	100
Redundant skin fold of neck	13	100
Brushfield spots	5	83
Cataract/cloudy cornea	30	86
Glaucoma	7	58
Abnormal retinal pigmentation	6	40
Optic disc pallor	17	74
Severe hypotonia	94	99
Abnormal Moro response	26	100
Hyporeflexia or areflexia	56	98
Poor sucking	74	96
Gavage feeding	26	100
Epileptic seizures	56	92
Intellectual disability	45	100
Impaired hearing	9	40
Nystagmus	30	81

From Heymans HAS. Cerebro-hepato-renal (Zellweger) syndrome: Clinical and biochemical consequences of peroxisomal dysfunctions. Thesis, University of Amsterdam, 1984.

impaired, but accurate assessment is limited, usually by the presence of both vision and hearing impairment. Almost all have some degree of sensorineural hearing loss and pigmentary degeneration of the retina. They have moderately dysmorphic features that may include epicanthal folds, a flat nose bridge, and low-set ears. Early hypotonia and hepatomegaly with impaired function are common. Levels of plasma cholesterol and high-density and low-density lipoprotein are often moderately reduced. Chondrodysplasia punctata and renal cortical cysts are absent. Postmortem study in these mild PBD-ZSD cases reveals micronodular liver cirrhosis and small, hypoplastic adrenals. The brain shows no malformations, except for severe hypoplasia of the cerebellar granule layer and ectopic locations of the Purkinje cells in the molecular layer.

Some patients with PBD-ZSD have milder and atypical phenotypes. They may present with peripheral neuropathy or with retinopathy, impaired vision, or cataracts in childhood, adolescence, or adulthood and have been considered to have Charcot-Marie-Tooth disease or Usher syndrome. Some patients with *PEX16* variants present with cerebellar ataxia. Some patients have survived to the fifth decade.

Rhizomelic Chondrodysplasia Punctata

RCDP is characterized by the presence of stippled foci of calcification within the hyaline cartilage and is associated with short stature, cataracts (72%), and multiple malformations caused by contractures. Vertebral bodies have coronal clefting filled by cartilage that results from an embryonic arrest. Disproportionate short stature affects the proximal parts of the extremities (rhizomelia; Fig. 106.4A). Radiologic abnormalities consist of shortening of the proximal limb bones, metaphyseal cupping, and disturbed punctate ossification (Fig. 106.4B). Height, weight, and head circumference are less than the third percentile, and these children have a severe intellectual disability. Skin changes such as those observed in ichthyosiform erythroderma are present in approximately 25% of patients. RCDP can be caused by pathogenic variants in one of four genes *(PEX5, PEX7, AGPS, GNPAT)*. Defects in *PEX7*, which most frequently lead to the RCDP phenotype, may also lead to a milder phenotype with clinical manifestations similar to those of adult Refsum disease, a later-onset disorder described later.

Isolated Defects of Peroxisomal Fatty Acid Oxidation

In the group of single-enzyme defects, acyl-CoA oxidase and bifunctional enzyme deficiency involve a single enzymatic step in peroxisomal fatty acid oxidation. **Defects of bifunctional enzyme** are found in approximately 15% of patients who are initially suspected of having PBD-ZSD. Patients with isolated **acyl-CoA oxidase deficiency** have a somewhat milder phenotype that resembles mild or intermediate PBD-ZSD or Usher syndrome and typically comes to attention because of the development of an early childhood leukodystrophy.

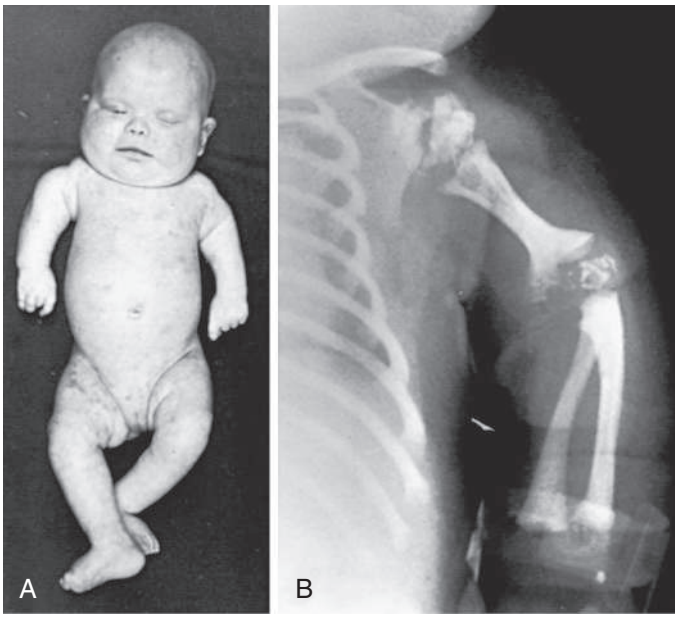

Fig. 106.4 A, Newborn infant with rhizomelic chondrodysplasia punctata. Note the severe shortening of the proximal limbs, the depressed bridge of the nose, hypertelorism, and widespread scaling skin lesions. B, Note the marked shortening of the humerus and epiphyseal stippling at the shoulder and elbow joints. *(Courtesy John P. Dorst, MD.)*

Isolated Defects of Plasmalogen Synthesis

Plasmalogens are lipids in which the first of three glycerol carbons is linked to an alcohol rather than a fatty acid. They are synthesized through a complex series of reactions, the first two steps of which are catalyzed by the peroxisomal enzymes dihydroxyacetone phosphate alkyl transferase (DHAPT, encoded by the gene *GNPAT*) and synthase (AGPS, encoded by the gene *AGPS*). Deficiency of either of these enzymes leads to RCDP types 2 and 3, respectively, phenotypes that are clinically indistinguishable from each other and from the peroxisomal import disorder RCDP1. This latter disorder is caused by a defect in *PEX7,* the receptor for PTS2 upon which DHAPT and AGPS are dependent for peroxisomal import. RCDP1 shares the severe deficiency of plasmalogens with these single-enzyme disorders but also has defects of phytanic oxidation. The fact that these single genetic disorders are associated with the full phenotype of RCDP suggests that a deficiency of plasmalogens is sufficient to produce it.

Adult (Classic) Refsum Disease

The defective enzyme (phytanoyl-CoA hydroxylase, encoded by the *PHYH* gene) is localized to the peroxisome. The manifestation of Refsum disease includes impaired vision from retinitis pigmentosa, anosmia, ichthyosis, peripheral neuropathy, ataxia, and occasionally cardiac arrhythmias. In contrast to infantile Refsum disease, cognitive function is normal, and there are no congenital malformations. Refsum disease often does not manifest until young adulthood, but visual disturbances such as night blindness, ichthyosis, and peripheral neuropathy may already be present in childhood and adolescence. Early diagnosis is important because institution of a phytanic acid–restricted diet can reverse the peripheral neuropathy and prevent the progression of the visual and central nervous system (CNS) manifestations. Adult Refsum disease may also be caused by defects in *PEX7*.

2-Methylacyl-CoA Racemase Deficiency (AMACR)

This disorder is caused by an enzyme defect that leads to the accumulation of the branched-chain fatty acids (phytanic and pristanic acid) and bile acids. Individuals present with typically an adult-onset peripheral neuropathy and may also have pigmentary degeneration of the retina.

Atypical Autosomal Dominant Disorders

Peroxisomal disorders are classically autosomal recessive in family pedigrees. X-linked adrenoleukodystrophy is a key exception. Several other exceptions of rare dominant inheritance of peroxisomal disease manifesting as neurologic disease have emerged for a few peroxisomal genes, including *ACOX1, PEX6*, and *DNM1L*.

Laboratory Findings

Clinical suspicion would be followed by specific biochemical determination of an abnormality and then confirmation through genetic testing targeted to specific genes. However, an extended gene panel and exome and genome testing have been used to identify pathogenic variants or variants of uncertain significance (VUS) in peroxisomal genes. *These patients still require biochemical testing for confirmation.* In addition, newborn screening for X-linked ALD using dried blood spots on filter paper in states across the United States and many parts of Europe also identifies other forms of peroxisomal deficiency. Early recognition of peroxisomal disorders in newborns will be a clinical reality.

Whether based on clinical suspicion, an abnormal newborn screen, or reported genetic variants, the biochemical characterization of peroxisomal disorders is a necessary step and uses the generally available testing strategy listed in Table 106.5. Measurement of plasma VLCFA levels is the most common assay. It must be emphasized that although plasma VLCFA levels are elevated in many patients with peroxisomal disorders and the same defect is the indirect basis for the newborn screen assay, this is not always the case. The most important exception is RCDP, in which VLCFA levels are normal but plasma phytanic acid levels are increased and red blood cell (RBC) plasmalogen levels are reduced. In other peroxisomal disorders, the biochemical abnormalities are still more restricted. Therefore a panel of tests is recommended and includes plasma levels of VLCFAs and phytanic, pristanic, and pipecolic acids and RBC levels of plasmalogens. Tandem mass spectrometry techniques also permit convenient quantitation of bile acids in plasma and urine. This panel of tests can be performed on very small amounts of venous blood and permits detection of most peroxisomal disorders.

Definition of the molecular defect either through gene panel testing or exome sequencing in the *proband* is essential for carrier detection and speeds prenatal diagnosis. Characterization of the pathogenic variant may be of prognostic value in patients with *PEX1* defects. This defect is present in approximately 60% of PBD patients, and about half the *PEX1* defects have the G843D allele, which is associated with a significantly milder phenotype than found in other pathogenic variants.

Diagnosis

Several noninvasive laboratory tests permit precise and early diagnosis of peroxisomal disorders (see Table 106.5). The challenge in PBDs is to differentiate them from the large variety of other conditions that can cause hypotonia, seizures, failure to thrive, or dysmorphic features. Experienced clinicians readily recognize classic Zellweger syndrome by its clinical manifestations. However, more mildly affected PBD-ZSD patients often do not show the full clinical spectrum of disease and may be identifiable only by laboratory assays. Clinical features that warrant diagnostic assessment include intellectual disability; weakness and hypotonia; dysmorphic features; neonatal seizures; retinopathy, glaucoma, or cataracts; hearing deficits; enlarged liver and impaired liver function; and chondrodysplasia punctata. The presence of one or more of these abnormalities increases the likelihood of this diagnosis. Atypical milder forms presenting as peripheral neuropathy have also been described.

Some patients with the isolated defects of peroxisomal fatty acid oxidation resemble those with ZSD and can be detected by the demonstration of abnormally high levels of VLCFAs.

Patients with RCDP must be distinguished from patients with *other* causes of chondrodysplasia punctata. RCDP is suspected clinically because of the shortness of limbs, developmental delays, and ichthyosis. The most decisive laboratory test is the demonstration of abnormally low plasmalogen levels in RBCs and an alteration in *PEX7*.

Table 106.5 Diagnostic Biochemical Abnormalities in Peroxisomal Disorders

DISORDER	VLCFA	PHYTANIC ACID	PRISTANIC ACID	PLASMALOGENS
PBD-ZSD	↑↑	↑*	↑*	↓
RCDP	Nl	↑	Nl	↓↓
ALD	↑	Nl	Nl	Nl
ACoX	↑	Nl	Nl	Nl
Bifunctional enzyme deficiency	↑	↑	↑	Nl
AMACR	Nl	↑	↑	Nl
Refsum disease	Nl	↑	↑	Nl

*Phytanic acid and pristanic acid accumulation is age dependent, and normal (Nl) levels may be seen in infants and young children.
VLCFA, Very long chain fatty acids; ZSD, Zellweger spectrum disorder; RCDP, rhizomelic chondroplasia punctata; ALD, adrenoleukodystrophy; ACoX, acyl-CoA oxidase deficiency; AMACR, 2-methylacyl-CoA racemase deficiency.

Complications
Patients with severe PBD-ZSD have multiple disabilities involving muscle tone, swallowing, cardiac abnormalities, liver disease, and seizures. These conditions are treated symptomatically, but the prognosis is poor, and most patients succumb in the first years of life. Similarly, individuals with RCDP have multiple systemic and neurologic issues. In addition, they may develop spinal cord compression at any level of the spine.

Treatment
The most effective therapy is the dietary treatment of adult Refsum disease with a phytanic acid–restricted diet. However, this only applies to this specific condition.

For patients with the somewhat milder variants of the peroxisome import disorders, success has been achieved with multidisciplinary early intervention, including physical and occupational therapy, hearing aids or cochlear implants, augmentative and alternative communication, nutrition, and support for the families. Although most patients continue to function in the impaired range, some make significant gains in self-help skills, and several are in stable condition in their teens or even early 20s.

Attempts to mitigate some of the secondary biochemical abnormalities include the oral administration of docosahexaenoic acid (DHA). The DHA level is greatly reduced in patients with disorders of peroxisome biogenesis, and this therapy normalizes DHA plasma levels. Although there were anecdotal reports of clinical improvement with DHA therapy, a randomized placebo-controlled study failed to find benefit.

Genetic Counseling
Most of these disorders can be diagnosed prenatally. Prenatal testing using chorionic villus sampling or amniocentesis enables genetic testing when the alteration is known, but biochemical measurements may be made using the same tests as described for postnatal diagnosis (see Table 106.5). Because of the 25% recurrence risk with autosomal recessive inheritance, couples with an affected child should be advised about the availability of prenatal and preconception diagnosis.

ADRENOLEUKODYSTROPHY
ALD is an X-linked disorder associated with the accumulation of saturated VLCFAs and a progressive dysfunction of the adrenal cortex and nervous system. It is the most common peroxisomal disorder.

Etiology
The key biochemical abnormality in ALD is the tissue accumulation of saturated VLCFAs, with a carbon chain length of 24 or more. Excess hexacosanoic acid ($C_{26:0}$) is the most striking and characteristic feature. This accumulation of fatty acids is caused by genetically deficient peroxisomal degradation of fatty acid. The defective gene *(ABCD1)* codes for a peroxisomal membrane protein (ALDP, the ALD protein). Many alterations in *ABCD1* have been determined to be pathogenic, with over half these being private or unique to the family. There is no genotype-phenotype correlation, as wide ranges of clinical severity can occur within a family across multiple individuals with the same pathogenic variant. A curated database of pathogenic variants is maintained (www.x-ald.nl). The mechanism by which the ALDP defect leads to VLCFA accumulation appears to be a disruption of transport of saturated fatty acids into the peroxisome, with resultant continued progressive elongation of fatty acids.

Epidemiology
The incidence of ALD in males is 1 in 21,000, and the combined incidence of ALD males and heterozygous females in the general population is estimated to be 1 in 17,000. All ethnicities are affected. The various phenotypes often occur in members of the same kindred.

Pathology
Characteristic lamellar cytoplasmic inclusions can be demonstrated on electron microscopy in adrenocortical cells, testicular Leydig cells, and nervous system macrophages. These inclusions probably consist of cholesterol esterified with VLCFA. They are most prominent in cells of the zona fasciculata of the adrenal cortex, which at first are distended with lipids and subsequently atrophy.

The nervous system displays two types of ALD lesions. In the severe cerebral form, demyelination is associated with an inflammatory response manifested by the accumulation of perivascular lymphocytes that is most intense in the involved region. In the slowly progressive adult form, **adrenomyeloneuropathy** (AMN), the main finding is a distal axonopathy that affects the long tracts in the spinal cord. In this form the inflammatory response is mild or absent.

Pathogenesis
The adrenal dysfunction is probably a direct consequence of the accumulation of VLCFAs. The cells in the adrenal zona fasciculata are distended with abnormal lipids. Cholesterol esterified to VLCFA is relatively resistant to adrenocorticotropic hormone (ACTH)–stimulated cholesterol ester hydrolases, and this limits the capacity to convert cholesterol to active steroids. In addition, $C_{26:0}$ excess increases the viscosity of the plasma membrane, which may interfere with receptor and other cellular functions.

There is no defined correlation between the neurologic phenotype and the nature of the pathogenic variant or the severity of the biochemical defect as assessed by plasma VLCFA levels or between the degree of adrenal involvement and nervous system involvement. The severity of the illness and rate of progression correlate with the intensity of the inflammatory response. The inflammatory response may be partially cytokine mediated and may involve an autoimmune response triggered in an unknown way by the excess of VLCFAs. Mitochondrial damage and oxidative stress also appear to contribute. Approximately half the patients do not experience the inflammatory response, although the basis of this difference is not understood.

Clinical Manifestations

There are five relatively distinct ALD phenotypes, three of which present in childhood with symptoms and signs. In all the phenotypes, development is usually normal in the first 3-4 years of life.

In the **childhood cerebral form** of ALD, symptoms most often are first noted between ages 4 and 8 years. The most common initial manifestations are hyperactivity, inattention, and worsening school performance in a child who had previously been a good student. *Auditory discrimination* is often impaired, although tone perception is preserved. This may be evidenced by difficulty in using the telephone and greatly impaired performance on intelligence tests in items that are presented verbally. Spatial orientation is often impaired. Other initial symptoms are disturbances of vision, ataxia, poor handwriting, seizures, and strabismus. Visual disturbances are often caused by involvement of the parietooccipital cortex rather than eye or optic tract abnormalities, which leads to variable and seemingly inconsistent visual capacity. Seizures occur in most patients and may represent the first manifestation of the disease. Some patients present with increased intracranial pressure. Impaired cortisol response to ACTH stimulation is present in 85% of patients, and mild hyperpigmentation is noted. In most patients with this phenotype, adrenal dysfunction is recognized only after the condition is diagnosed because of the cerebral symptoms. Cerebral childhood ALD tends to progress rapidly with increasing spasticity and paralysis, visual and hearing loss, and loss of ability to speak or swallow. The mean interval between the first neurologic symptom and an apparently unresponsive wakeful state is 1.9 years. Patients may continue in this apparently unresponsive wakeful state for ≥10 years.

Adolescent ALD designates patients who experience neurologic symptoms between ages 10 and 21 years. The manifestations resemble those of childhood cerebral ALD except that progression is slower. Approximately 10% of patients present acutely with status epilepticus, adrenal crisis, acute encephalopathy, or coma.

AMN first manifests in late adolescence or adulthood as a progressive paraparesis caused by long tract degeneration in the spinal cord. Approximately half the affected males also have involvement of the cerebral white matter.

The **Addison-only** phenotype is an important condition. Of male patients with Addison disease, 25% may have the biochemical defect of ALD. Many of these patients have intact neurologic systems, whereas others have subtle neurologic signs. Many acquire AMN in adulthood.

The term **asymptomatic ALD** is applied to persons who have the biochemical defect of ALD but are free of neurologic or endocrinal disturbances. Almost all persons with the gene defect eventually become neurologically symptomatic.

Approximately 50% of female heterozygotes acquire a syndrome that resembles AMN but is milder and of later onset. Adrenal insufficiency and cerebral disease are rare.

Cases of typical ALD have occurred in relatives of those with AMN. One of the most difficult problems in the management of ALD is the common observation that affected individuals in the same family may have quite different clinical courses. For example, in one family, an affected male may have severe classic ALD culminating in death by age 10 years, and another brother will have the later-onset AMN.

Laboratory and Radiographic Findings

The most specific and important laboratory finding is the demonstration of abnormally high levels of VLCFAs in plasma, RBCs, or cultured skin fibroblasts. Positive results are obtained in all male patients with ALD and in approximately 85% of female carriers of ALD. Pathogenic variant analysis is the most reliable method for the identification of carriers. Simply finding a variation in ABCD1 is not adequate for making the diagnosis of ALD. It must be shown to segregate with elevated VLCFA levels.

Neuroimaging

Patients with childhood cerebral or adolescent ALD have characteristic white matter lesions on MRI. In 80% of patients, the lesions are symmetric and involve the splenium of the corpus callosum and periventricular white matter in the posterior parietal and occipital lobes. Many will show a garland of contrast enhancement adjacent and anterior to the posterior hypodense lesions (Fig. 106.5). This zone corresponds to the zones of intense perivascular lymphocytic infiltration where the blood-brain barrier breaks down. In 10–15% of patients, the initial lesions are frontal. Unilateral lesions that produce a mass effect suggestive of a brain tumor may occur rarely. MRI provides a clearer delineation of normal and abnormal white matter than does CT and is the preferred imaging modality.

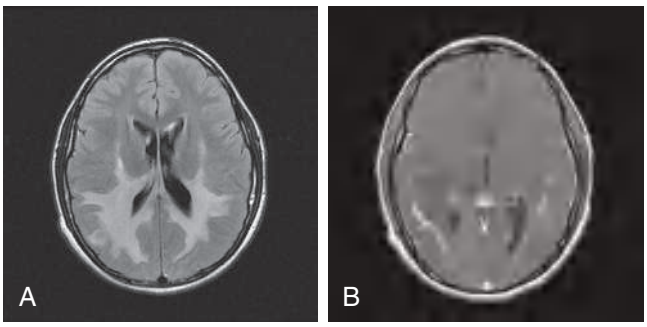

Fig. 106.5 Characteristic MRI findings in cerebral adrenoleukodystrophy. **A,** Symmetric T2-weighted MRI abnormalities involve the posterior white matter, including the corpus callosum. **B,** Contrast administration reveals a garland of enhancement.

Impaired Adrenal Function

More than 85% of patients with the childhood form of ALD have elevated levels of ACTH in plasma and a subnormal rise of cortisol levels in plasma after IV injection of ACTH.

Newborn Screening and Diagnosis

Diagnosis of asymptomatic males is available by newborn screening, which allows for early diagnosis of ALD years before the manifestations of disease. Males then enter a program of surveillance for adrenal insufficiency and early detection of potential cerebral disease. Females identified through these programs should also have confirmatory testing, genetic counseling for the family, and screening of other at-risk males. Females do not generally require any other monitoring in childhood. This early screening paradigm allows for early surveillance of ALD neurologic symptoms, which are difficult to distinguish from the more common attention-deficit disorders or learning disabilities of school-age children. Early diagnosis could lead to early cortisol treatment for adrenal problems, which could be life-threatening. Early bone marrow transplant is likely to result as a benefit of screening. For positive newborn screens or for individuals with clinical suspicion of ALD, confirmatory VLCFA testing and genetic counseling should be provided.

The Earliest Manifestations of Childhood Cerebral ALD

Rapid progression, signs of dementia, or difficulty in auditory discrimination suggest ALD. Even in early stages, neuroimaging shows abnormal changes. Other leukodystrophies or multiple sclerosis may sometimes mimic these radiographic findings, although early ALD has more of a predilection for the posterior brain than its mimics. Definitive diagnosis depends on demonstration of VLCFA excess, which occurs only in ALD and the other peroxisomal disorders.

Cerebral forms of ALD, especially if asymmetric, may be misdiagnosed as gliomas or other mass lesions. Individuals have received brain biopsy and, rarely, other therapies before the correct diagnosis was made. Measurement of VLCFAs in plasma is the most reliable differentiating test.

Adolescent or adult cerebral ALD can be confused with psychiatric disorders, dementing disorders, multiple sclerosis, or epilepsy. The first clue to the diagnosis of ALD may be the demonstration of characteristic white matter lesions by neuroimaging; VLCFA assays are confirmatory.

ALD *cannot* be distinguished clinically from other forms of Addison disease; it is recommended that assays of VLCFA levels be performed

in all male patients with Addison disease. ALD patients *do not* usually have antibodies to adrenal tissue in their plasma.

Complications

An avoidable complication is the occurrence of adrenal insufficiency. The most difficult neurologic problems are those related to bed rest, contracture, coma, and swallowing disturbances. Other complications involve behavioral disturbances and injuries associated with defects of spatial orientation, impaired vision and hearing, and seizures.

Treatment

Corticosteroid replacement for adrenal insufficiency or adrenocortical hypofunction is effective. It may be lifesaving and may increase general strength and well-being, but it does not alter the course of the neurologic disability.

Bone Marrow Transplantation

Bone marrow transplantation (BMT) or hematopoietic stem cell therapy (HSCT) benefits patients who show early evidence of the inflammatory demyelination characteristic of the rapidly progressive neurologic disability in young males and adolescents with the cerebral ALD phenotype. BMT carries risk, and patients must be evaluated and selected with care. The mechanism of the beneficial effect is incompletely understood. Bone marrow–derived cells do express ALDP, the protein that is deficient in ALD; approximately 50% of brain microglial cells are bone marrow derived. The favorable effect may be caused by modification of the brain inflammatory response. Follow-up of young males and adolescents who had early cerebral involvement has shown stabilization. On the other hand, BMT does not arrest the course in those who already had severe brain involvement and may accelerate disease progression under these circumstances. The ALD MRI score and the use of performance measures on IQ testing have shown some predictive ability for boys likely to benefit from this procedure. Transplant is not recommended in patients with performance IQ significantly <80. Unfortunately, in more than half the patients who are diagnosed because of neurologic symptoms, the illness is so advanced at diagnosis that they are not candidates for transplant.

Consideration of BMT is most relevant in neurologically asymptomatic or mildly involved patients. Screening at-risk relatives of symptomatic patients identifies these patients most frequently. Screening by measurement of plasma VLCFA levels in patients with Addison disease may also identify candidates for BMT. Because of its risk (10–20% mortality) and because up to 50% of untreated patients with ALD do not develop inflammatory brain demyelination, transplant is not recommended in patients who are free of demonstrable brain involvement on MRI. MRI is also of key importance for the crucial decision of whether transplant should be performed. MRI abnormalities precede clinically evident neurologic or neuropsychologic abnormalities. The brain MRI should be monitored at 6-month intervals in neurologically asymptomatic young males and adolescents age 3-15 years. If the MRI is normal, BMT is not indicated. If brain MRI abnormalities develop, the young male should be evaluated by a center familiar with transplant for ALD. This should include MRI, neurologic, and neuropsychologic evaluations. It is not known whether BMT has a favorable effect on the noninflammatory spinal cord involvement in adults with the adrenomyeloneuropathy phenotype.

Autologous hematopoietic stem cell gene therapy **(elivaldogene autotemcel)** is approved for patients 4-17 years of age to slow the neurologic progression of early, active cerebral adrenoleukodystrophy.

Supportive Therapy

The progressive behavioral and neurologic disturbances associated with the childhood form of ALD are extremely difficult for the family. ALD patients require the establishment of a comprehensive management program and partnership among the family, physician, visiting nursing staff, school authorities, and counselors. In addition, parent support groups (e.g., United Leukodystrophy Foundation) are often helpful. Communication with school authorities is important because under the provisions of Public Law 94-142, children with ALD qualify for special services as "other health impaired" or "multi-handicapped." Depending on the rate of progression of the disease, special needs might range from relatively low-level resource services within a regular school program to home- and hospital-based teaching programs for children who are not mobile.

Management challenges vary with the stage of the illness. The early stages are characterized by subtle changes in affect, behavior, and attention span. Counseling and communication with school authorities are of prime importance. Changes in the sleep–wake cycle can be benefited by the judicious use of nighttime sleep medications.

As the leukodystrophy progresses, the modulation of muscle tone and support of bulbar muscular function are major concerns. Baclofen in gradually increasing doses (5 mg twice a day to 25 mg four times a day) is an effective pharmacologic agent for the treatment of acute episodic painful muscle spasms. Other agents may also be used, with care taken to monitor the occurrence of side effects and drug interactions. As the leukodystrophy progresses, bulbar muscular control is lost. Although initially this can be managed by changing the diet to soft and pureed foods, most patients eventually require a gastrostomy tube. At least 30% of patients have focal or generalized seizures that usually readily respond to standard anticonvulsant medications.

Genetic Counseling and Prevention

Genetic counseling and appropriate monitoring are of crucial importance. Extended-family screening should be offered to all at-risk relatives of symptomatic patients; one program led to the identification of >250 asymptomatic affected males and 1,200 women heterozygous for ALD. The plasma assay permits reliable identification of affected males in whom plasma VLCFA levels are increased already on the day of birth. Identification of asymptomatic males permits institution of steroid replacement therapy when appropriate and prevents adrenal crisis, which may be fatal. Monitoring of brain MRI also permits identification of patients who are candidates for BMT at a stage when this procedure has the greatest chance of success. Plasma VLCFA assay is recommended in all male patients with Addison disease. ALD has been shown to be the cause of adrenal insufficiency in >25% of boys with Addison disease of unknown cause. Identification of women heterozygous for ALD is more difficult than that of affected males. Plasma VLCFA levels are normal in 15–20% of heterozygous women, and failure to note this has led to serious errors in genetic counseling. DNA analysis permits accurate identification of carriers, provided that the pathogenic variant has been defined in a family member, and this is the procedure recommended for the identification of heterozygous women.

Prenatal diagnosis of affected male fetuses can be achieved by determination of the known pathogenic variant or by the measurement of VLCFA levels in cultured amniocytes or chorionic villus cells. Whenever a new patient with ALD is identified, a detailed pedigree should be constructed and efforts made to identify all at-risk female carriers and affected males. These investigations should be accompanied by careful and sympathetic attention to social, emotional, and ethical issues during counseling.

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106.3 Disorders of Lipoprotein Metabolism and Transport

Lee A. Pyles

EPIDEMIOLOGY OF BLOOD LIPIDS AND CARDIOVASCULAR DISEASE

There is a strong association between average intake of saturated fats, plasma cholesterol, and mortality from coronary heart disease (CHD). Of all common chronic diseases, none is so clearly influenced by both environmental *and* genetic factors as CHD. This multifactorial disorder is strongly associated with increasing age and male gender, although it is increasingly apparent that heart disease is underrecognized in women. Tobacco use confers a twofold higher lifetime risk.

Sedentary activity and high intake of processed sugars leading to adiposity increase risk through differences in the plasma levels of atherogenic lipoproteins. Family history reflects the combined influence of lifestyle and genetic predisposition to early heart disease. Risk of premature heart disease associated with positive family history is 1.7 times higher than in families with no such history.

Atherosclerosis begins during childhood. The Johns Hopkins Precursors Study demonstrated that White male medical students with blood cholesterol levels in the lowest quartile showed only a 10% incidence of CHD 3 decades later, whereas those in the highest quartile had a 40% incidence. The Pathobiological Determinants of Atherosclerosis in Youth Study demonstrated a significant relationship between the weight of the abdominal fat pad and the extent of atherosclerosis found at autopsy on individuals 15-34 years of age. The Bogalusa Heart Study of more than 3,000 Black and White children and adolescents has provided the most comprehensive longitudinal data relating the presence and severity of CHD risk factors with semi-quantifiable severity of atherosclerosis. Coronary atherosclerosis was present in 8.5% of military autopsies performed after combat or unintentional injuries.

The *fetal origins hypothesis* is based on the observation that infants born with low birthweight have a higher incidence of heart disease as adults. Epidemiologic studies support the idea that prenatal and early postnatal conditions may affect adult health status. Children who are large for gestational age at birth and exposed to an intrauterine environment of either diabetes or maternal obesity are at increased risk of eventually developing **metabolic syndrome** (insulin resistance, type 2 diabetes, obesity, CHD). Breastfeeding preterm infants confers a long-term cardioprotective benefit 13-16 years later. Those adolescents who were breastfed as infants had lower C-reactive protein (CRP) concentrations and a 14% lower low-density lipoprotein (LDL)/high-density lipoprotein (HDL) ratio than formula-fed infants. The impact of early nutrition and other lifestyle variables on gene expression via *epigenetics* is one mechanism by which adult metabolism and body composition may be influenced.

Secondary causes of hyperlipidemia may be the result of drugs (cyclosporine, corticosteroids, isotretinoin, protease inhibitors, alcohol, thiazide diuretics, β-blocking agents, valproate) or various diseases (nephrotic syndrome, hypothyroidism, Cushing syndrome, anorexia nervosa, obstructive jaundice). Psychotropic medications, including second-generation antipsychotics such as olanzapine, are also associated with dyslipidemia, obesity, and insulin resistance.

BLOOD LIPIDS AND ATHEROGENESIS

Numerous epidemiologic studies demonstrate the association of hypercholesterolemia, referring to elevated total, non-HDL, and LDL blood cholesterol, with atherosclerotic disease. Atherosclerosis affects primarily the coronary arteries but may also involve the aorta, arteries of the lower extremities, and carotid arteries.

The early stage of development of atherosclerosis is thought to begin with vascular endothelial dysfunction and intima-media thickness, which has been shown to occur in preadolescent children with risk factors such as obesity or familial hypercholesterolemia. The complex process of penetrating the intimal lining of the vessel may result from a variety of insults, including the presence of highly toxic oxidized LDL particles. Lymphocytes and monocytes penetrate the damaged endothelial lining, where they become macrophages laden with LDL lipids and then become foam cells. Such accumulation is counterbalanced by HDL particles capable of removing lipid deposits from the vessel wall. Fundamental to plaque formation is an inflammatory process (elevated CRP) involving macrophages and the arterial wall. The deposition of lipid within the subendothelial lining of the arterial wall appears macroscopically as fatty streaks, which may to some degree be reversible. A later stage of plaque development involves disruption of arterial smooth muscle cells stimulated by the release of tissue cytokines and growth factors. The *atheroma* is composed of a core of fatty substance separated from the lumen by collagen and smooth muscle (Fig. 106.6). Growth of the atherosclerotic plaque may result in ischemia of the tissue supplied by the artery. Chronic inflammation within the atheroma results in plaque instability and subsequent rupture. Platelet adherence leads to clot formation at the site of rupture, resulting in myocardial infarction (MI) or a cerebrovascular accident (CVA), depending on the site of thrombosis or thromboembolism.

PLASMA LIPOPROTEIN METABOLISM AND TRANSPORT

Lipoproteins are soluble complexes of lipids and proteins that effect transport of fat absorbed from the diet, or synthesis by the liver and adipose tissues, for utilization and storage. Dietary fat is transported from the small intestine as chylomicrons. Lipids synthesized by the liver as very low-density lipoproteins (VLDLs) are catabolized to intermediate-density lipoproteins (IDLs) and LDLs. HDL is fundamentally involved in VLDL and chylomicron metabolism and cholesterol transport. Nonesterified free fatty acids are metabolically active lipids derived from lipolysis of triglycerides stored in adipose tissue and bound to albumin for circulation in the plasma (Fig. 106.7).

Lipoproteins consist of a central core of triglycerides and cholesteryl esters surrounded by phospholipids, cholesterol, and proteins (Fig. 106.8). The density of the several classes of lipoproteins is inversely proportional to the ratio of lipid to protein, which is generally denser (Fig. 106.9).

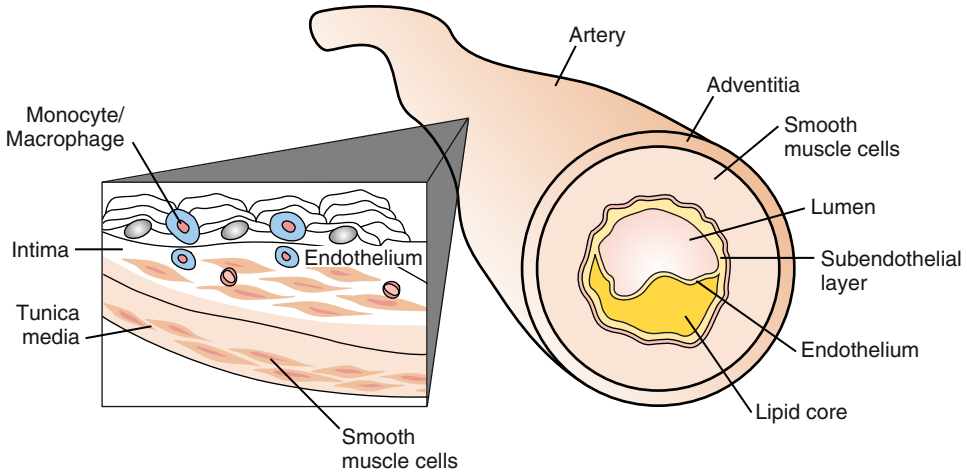


Fig. 106.6 The early stage of development of atherosclerosis begins with penetration of the intimal lining of the vessel by inflammatory cells. Deposition of lipid within the subendothelial lining of the arterial wall eventually leads to disruption of smooth muscle cells to form an atheromatous lipid core that impinges on the lumen. Chronic inflammation leads to plaque instability, setting the stage for plaque rupture and complete occlusion of the vessel lumen by clot formation.

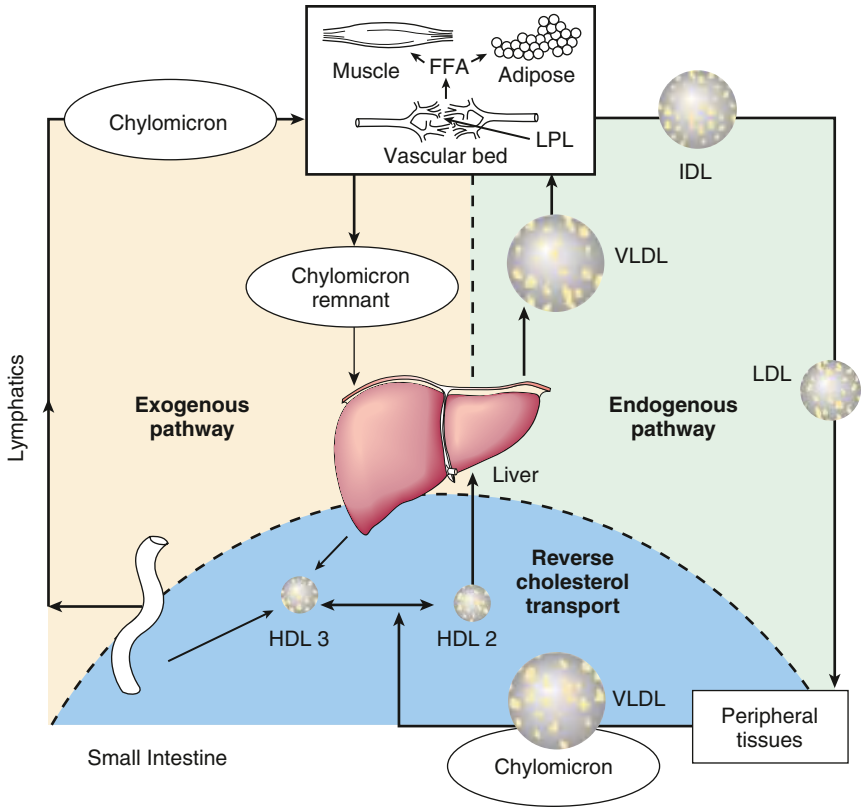


Fig. 106.7 The exogenous, endogenous, and reverse cholesterol pathways. The exogenous pathway transports dietary fat from the small intestine as chylomicrons to the periphery and the liver. The endogenous pathway denotes the secretion of very low-density lipoprotein (VLDL) from the liver and its catabolism to intermediate-density lipoprotein (IDL) and low-density lipoprotein (LDL). Triglycerides are hydrolyzed from the VLDL particle by the action of lipoprotein lipase (LPL) in the vascular bed, yielding free fatty acids (FFAs) for utilization and storage in muscle and adipose tissue. High-density lipoprotein (HDL) metabolism is responsible for the transport of excess cholesterol from the peripheral tissues back to the liver for excretion in the bile. Nascent HDL-3 particles derived from the liver and small intestine are esterified to more mature HDL-2 particles by enzyme-mediated movement of chylomicron and VLDL into the HDL core, which is removed from the circulation by endocytosis.

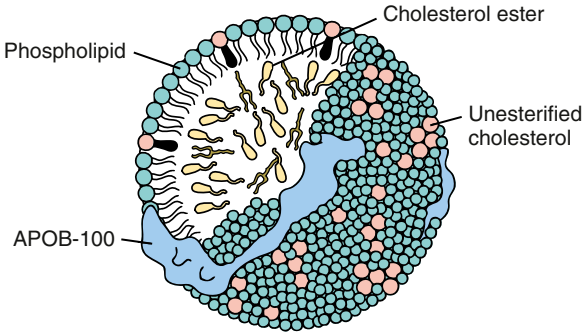


Fig. 106.8 Schematic of low-density lipoprotein. Lipoprotein consists of a central core of cholesteryl esters surrounded by phospholipids, cholesterol, and protein.

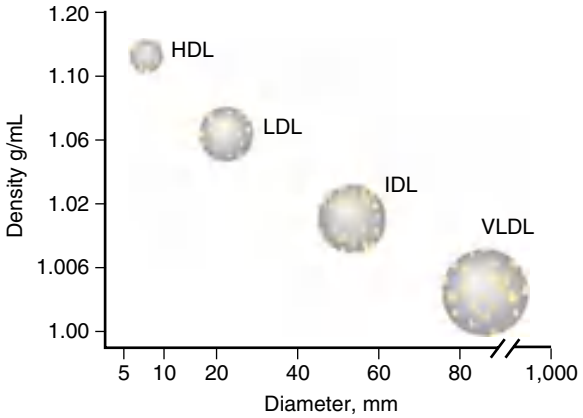


Fig. 106.9 The density of the several classes of lipoprotein is inversely proportional to the ratio of lipid to protein. As lipid is less dense than protein, the more lipid contained in the particle increases its size and decreases its density. HDL, High-density lipoprotein; LDL, low-density lipoprotein; IDL, intermediate-density lipoprotein; VLDL, very low-density lipoprotein.

Constituent proteins known as *apolipoproteins* are responsible for a variety of metabolic functions in addition to their structural role, including as cofactors or inhibitors of enzymatic pathways and mediators of lipoprotein binding to cell surface receptors (Table 106.6). **ApoA** is the major apolipoprotein (Apo) of HDL. **ApoB** is present in LDL, VLDL, IDL, and chylomicrons. ApoB-100 is derived from the liver, whereas apoB-48 comes from the small intestine. ApoC-I, C-II, and C-III are small peptides important in triglyceride metabolism. Loss of function and disruptive pathogenic variants of the *APOC3* gene are associated with low levels of triglycerides and a reduced risk of ischemic CHD. Likewise, **apoE**, which is present in VLDL, HDL, chylomicrons, and chylomicron remnants, plays an important role in the clearance of triglycerides.

Transport of Exogenous (Dietary) Lipids

All dietary fat except medium-chain triglycerides is efficiently carried into the circulation by way of lymphatic drainage from the intestinal mucosa. Triglyceride and cholesteryl esters combine with apoA and apoB-48 in the intestinal mucosa to form chylomicrons, which are carried into the peripheral circulation via the lymphatic system. HDL particles contribute apoC-II to the chylomicrons, required for the activation of *lipoprotein lipase* (LPL) within the capillary endothelium of adipose, heart, and skeletal muscle tissue. Free fatty acids are oxidized, esterified for storage as triglycerides, or released into the circulation bound to albumin for transport to the liver. After hydrolysis of the triglyceride core from the chylomicron, apoC particles are recirculated back to HDL. The subsequent contribution of apoE from HDL to the remnant chylomicron facilitates binding of the particle to the hepatic LDL receptor (LDL-R). Within the hepatocyte, the chylomicron remnant may be incorporated into membranes, resecreted as lipoprotein

Table 106.6 Characteristics of the Major Lipoproteins

LIPOPROTEIN	SOURCE	SIZE (nm)	DENSITY (g/mL)	COMPOSITION		
				PROTEIN (%)	LIPID (%)	APOLIPOPROTEINS
Chylomicrons	Intestine	80-1,200	<0.95	1-2	98-99	C-I, C-II, C-III, E, A-I, A-II, A-IV, B-48
Chylomicron remnants	Chylomicrons	40-150	<1.0006	6-8	92-94	B-48, E
VLDL	Liver, intestine	30-80	0.95-1.006	7-10	90-93	B-100, C-I, C-II, C-III
IDL	VLDL	25-35	1.006-1.019	11	89	B-100, E
LDL	VLDL	18-25	1.019-1.063	21	79	B-100
HDL	Liver, intestine VLDL, chylomicrons	5-20	1.125-1.210	32-57	43-68	A-I, A-II, A-IV C-I, C-II, C-III D, E

HDL, High-density lipoproteins; IDL, intermediate-density lipoproteins; LDL, low-density lipoproteins; VLDL, very low-density lipoproteins.

back into the circulation, or secreted as bile acids. Normally, all dietary fat is disposed of within 8 hours after the last meal, an exception being individuals with a disorder of chylomicron metabolism. **Postprandial hyperlipidemia** is a risk factor for atherosclerosis. Abnormal transport of chylomicrons and their remnants may result in their absorption into the blood vessel wall as *foam cells,* caused by the ingestion of cholesteryl esters by macrophages, the earliest stage in the development of fatty streaks.

Transport of Endogenous Lipids from the Liver

The formation and secretion of VLDL from the liver and its catabolism to IDL and LDL particles describe the *endogenous* lipoprotein pathway. Fatty acids used in the hepatic formation of VLDL are derived primarily by uptake from the circulation. VLDL appears to be transported from the liver as rapidly as it is synthesized, and it consists of triglycerides, cholesteryl esters, phospholipids, and apoB-100. Nascent particles of VLDL secreted into the circulation combine with apoC and apoE. The size of the VLDL particle is determined by the amount of triglyceride present, progressively shrinking in size as triglyceride is hydrolyzed by the action of LPL, yielding free fatty acids for utilization or storage in muscle and adipose tissue. Hydrolysis of approximately 80% of the triglyceride present in VLDL particles produces IDL particles containing an equal amount of cholesterol and triglyceride. The remaining remnant IDL is converted to LDL for delivery to peripheral tissues or to the liver. ApoE is attached to the remnant IDL particle to allow binding to the cell and subsequent incorporation into the lysosome. Individuals with a deficiency of either apoE2 or hepatic triglyceride lipase accumulate IDL in the plasma.

LDL particles account for approximately 70% of the plasma cholesterol in normal individuals. LDL receptors are present on the surfaces of nearly all cells. Most LDL is taken up by the liver, and the rest is transported to peripheral tissues such as the adrenal glands and gonads for steroid synthesis. Dyslipidemia is greatly influenced by LDL-R activity. The efficiency with which VLDL is converted into LDL is also important in lipid homeostasis. The normal newborn LDL level of 50 mg/dL is probably adequate for steroid synthesis throughout the life cycle.

High-Density Lipoprotein and Reverse Cholesterol Transport

Because hepatic secretion of lipid particles into the bile is the only mechanism by which cholesterol can be removed from the body, transport of excess cholesterol from the peripheral cells is a vitally important function of HDL. HDL is heavily laden with apoA-I–containing lipoproteins, which is nonatherogenic, in contrast to B lipoproteins. Cholesterol-poor nascent HDL particles secreted by the liver and small intestine are esterified to more mature HDL-2 particles by the action of the enzyme lecithin-cholesterol acyltransferase **(LCAT)**, which facilitates movement of chylomicrons and VLDL into the HDL core. HDL-2 may transfer cholesteryl esters back to apoB lipoproteins mediated by cholesteryl ester transfer protein (CETP), or the cholesterol-rich particle may be removed from the plasma by endocytosis, completing reverse cholesterol transport. Low HDL may be genetic (deficiency of apoA-I) or secondary to increased plasma triglyceride.

LCAT deficiency results in diminished maturation of HDL particles, affecting their ability to do reverse cholesterol transport. This reduces its protective effect on atherosclerosis. There are rare reports, however, of less-than-expected severity of atherosclerosis despite low HDL secondary to LCAT deficiency, suggesting that the relationship may, for unknown reasons, be variable.

HYPERLIPOPROTEINEMIAS

Hypercholesterolemia

See Table 106.7.

Familial Hypercholesterolemia

Familial hypercholesterolemia (FH) is a monogenic autosomal dominant disorder characterized by strikingly elevated LDL cholesterol (LDL-C), premature cardiovascular disease (CVD), and tendon xanthomas. It is predominantly associated with defects of LDL-R activity but also includes defects in the genes for apoB *(APOB)* and the proprotein convertase subtilisin/kexin type 9 (PCSK-9), a protein important in LDL endocytosis. Of the almost 1,200 *LDLR* pathogenic variants described, some result in failure of synthesis of the LDL-R (receptor negative) and others cause defective binding or release at the lipoprotein-receptor interface. Receptor-negative pathogenic variants result in more severe phenotypes than receptor-defective pathogenic variants. Data from the Netherlands has confirmed the importance of LDL reduction in FH; the major adverse coronary event risk for affected siblings with statin treatment begun at age 10 years mirrored that of unaffected siblings rather than the risk profile of the affected parent.

Homozygous Familial Hypercholesterolemia

FH homozygotes inherit two abnormal LDL receptor genes, resulting in markedly elevated plasma cholesterol levels ranging between 500 and 1,200 mg/dL. Triglyceride levels are normal to mildly elevated, and HDL levels may be slightly decreased. The condition occurs in 1 in 500,000 persons. Receptor-negative patients have <2% normal LDL-R activity, whereas those who are receptor defective may have as much as 25% normal activity and consequently a better prognosis.

However, the prognosis is poor regardless of the specific LDL-R aberration. Severe atherosclerosis involving the aortic root and coronary arteries is present by early to middle childhood. These children usually present with **xanthomas**, which may cause thickening of the Achilles tendon or extensor tendons of the hands, or cutaneous lesions on the hands, elbows, knees, or buttocks (Figs. 106.10-106.12). Corneal arcus may be present. Family history is informative because premature heart disease is strongly prevalent among relatives of both parents. The diagnosis may be confirmed genetically or by measuring LDL-R

Table 106.7 Hyperlipoproteinemias

DISORDER	LIPOPROTEINS ELEVATED	CLINICAL FINDINGS	GENETICS	ESTIMATED INCIDENCE
Familial hypercholesterolemia	LDL	Tendon xanthomas, CHD	AD	1 in 500
Familial defective ApoB-100	LDL	Tendon xanthomas, CHD	AD	1 in 1,000
Autosomal recessive hypercholesterolemia	LDL	Tendon xanthomas, CHD	AR	<1 in 1,000,000
Sitosterolemia	LDL	Tendon xanthomas, CHD	AR	<1 in 1,000,000
Polygenic hypercholesterolemia	LDL	CHD		1 in 30?
Familial combined hyperlipidemia	LDL, TG	CHD	AD	1 in 200
Familial dysbetalipoproteinemia	LDL, TG	Tuberoeruptive xanthomas, peripheral vascular disease	AD	1 in 10,000
Familial chylomicronemia (Frederickson type I)	TG ↑↑	Eruptive xanthomas, hepatosplenomegaly, pancreatitis	AR	1 in 1,000,000
Familial hypertriglyceridemia (Frederickson type IV)	TG ↑	±CHD	AD	1 in 500
Familial hypertriglyceridemia (Frederickson type V)	TG ↑↑	Xanthomas ± CHD	AD	—
Familial hepatic lipase deficiency	VLDL	CHD	AR	<1 in 1,000,000

AD, Autosomal dominant; AR, autosomal recessive; CHD, coronary heart disease; LDL, low-density lipoproteins, TG, triglycerides; VLDL, very low-density lipoproteins.

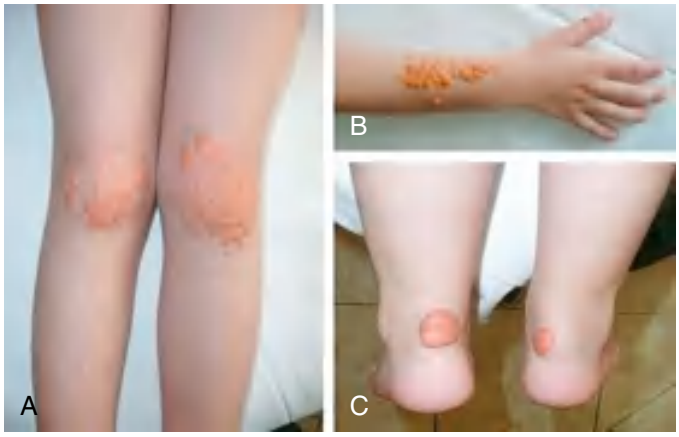


Fig. 106.10 Homozygous familial hypercholesterolemia. Tendon xanthomas in a 5-year-old boy with homozygous FH noted at the knee (**A**), wrist (**B**), and Achilles (**C**). *(Modified from Macchiaiolo M, Gagliardi MG, Toscano A, et al. Homozygous familial hypercholesterolaemia. Lancet. 2012;379:1330.)*

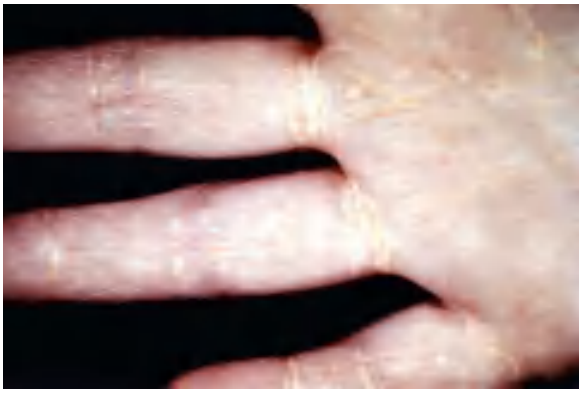


Fig. 106.11 Striate palmar xanthomata. *(From Durrington P. Dyslipidaemia, Lancet. 2003;362:717–731.)*

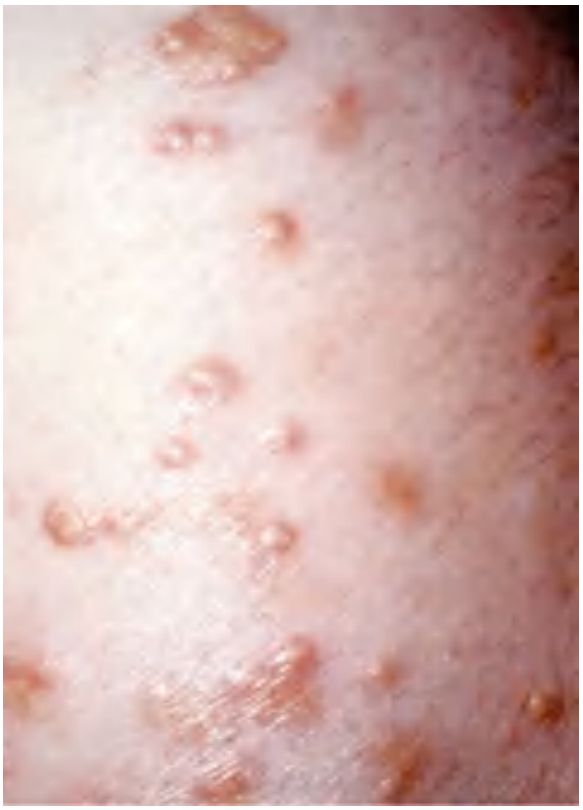


Fig. 106.12 Eruptive xanthomata on extensor surface of forearm. *(From Durrington P. Dyslipidaemia, Lancet. 2003;362:717–731.)*

activity in cultured skin fibroblasts. Phenotypic expression of the disease may also be assessed by measuring receptor activity on the surface of lymphocytes by using cell-sorting techniques.

Untreated homozygous patients rarely survive to adulthood. Symptoms of coronary insufficiency may occur early, and sudden death is common. LDL apheresis to remove LDL particles selectively from the

circulation is recommended for many children because it slows the progression of atherosclerosis. Liver transplantation is also successful in decreasing LDL-C levels, but complications related to immunosuppression are common. HMG-CoA reductase inhibitors may be modestly effective depending on the specific class of LDL-R defect present. Combination therapy with *ezetimibe,* selectively blocking cholesterol adsorption in the gut, usually results in further decline in LDL levels and has replaced the use of bile acid sequestrants. Clinical trials using microsomal triglyceride transfer protein inhibition with *lomitapide* (an oral agent) resulted in significant reduction of all apoB lipoproteins, including LDL, but hepatic fat deposition as a side effect limits this pharmacologic approach. *Mipomersen* (subcutaneous injection), an antisense oligonucleotide that binds to the apolipoprotein B mRNA, reduces the synthesis of apoB and thus also VLDL and LDL. LDL cholesterol levels may decline approximately 25% with this treatment. Adverse effects include flulike symptoms, hepatic steatosis, and cirrhosis.

Heterozygous Familial Hypercholesterolemia

Heterozygous FH is the most common single-gene disorder associated with acute coronary syndromes and atherosclerotic CHD in adults. Its prevalence is approximately 1 in 250 individuals worldwide, but the frequency may be greater in select populations, such as French Canadians, Afrikaners, Ashkenazi Jews, and Christian Lebanese, as a result of the founder effect of unique pathogenic variants. Heart disease accounts for more than half of all deaths in Western society, with the pathogenesis having both environmental and genetic influences.

Because heterozygous FH is a dominant condition with nearly full penetrance, 50% of first-degree relatives of affected individuals will have the disease, as will 25% of second-degree relatives. An estimated 20 million people have FH worldwide. Symptoms of CHD usually occur at the mean age of 45-48 years in males and a decade later in females. Genetic testing of both adult and pediatric patients who fulfill clinical diagnostic criteria for the diagnosis of heterozygous FH is variably positive dependent on the population under investigation.

One cannot overemphasize the importance of family history for suspecting the possibility of FH, especially given the low rate of cholesterol screening for children in primary care offices. Because the risk of CHD in individuals with FH can be up to 20 times greater than in the general population, guidelines have advocated for universal screening for cholesterol in childhood. There is also an interest in genetic testing for persons with suspected FH because of variability in phenotype based on genotype.

Plasma levels of LDL-C also do not allow unequivocal diagnosis of FH heterozygotes; values are generally twice normal for age because of one absent or dysfunctional allele. The U.S. MED-PED *(Make Early Diagnosis–Prevent Early Death)* Program has formulated diagnostic criteria.

Similar criteria with minor variations exist in the United Kingdom (Simon Broome criteria) and Holland (Dutch Lipid Clinic Network criteria). Within well-defined FH families, the diagnosis is reliably established according to LDL cutoff points. More stringent criteria are required to establish the diagnosis in previously undiagnosed families, requiring strong evidence of an autosomal inheritance pattern and higher LDL cutoff points. At a total cholesterol level of 310 mg/dL, only 4% of adults in the general population would have FH, whereas 95% of adults who were first-degree relatives of known cases would have the disease.

Very high cholesterol levels in children should prompt extensive screening of adult first- and second-degree relatives ("reverse cascade" cholesterol screening). In the general population, a child younger than age 18 years with total plasma cholesterol of 270 mg/dL and/or LDL-C of 200 mg/dL has an 88% chance of having FH (Table 106.8). *Formal clinical diagnosis of FH is based on the presence of two or more family members having elevated LDL-C levels* (the 95th percentile LDL-C level cutoff points for children vary with age and are lower than for adults; Table 106.9). The criteria for probable FH in a child whose first-degree relative has known FH require only modest elevation of total cholesterol to 220 mg/dL (LDL-C 160 mg/dL; see Table 106.8). The challenge of childhood FH diagnosis is heightened by the lack of clinical stigmata such as xanthomata that are employed in the Simon Broome and Dutch Lipid Clinic Network schema and highlights the needed shift toward genetic diagnosis.

Treatment of children with FH should begin with a rigorous low-fat diet. Diet alone is rarely sufficient for decreasing blood cholesterol levels to acceptable levels (LDL-C <130 mg/dL). Ezetimibe blocks cholesterol adsorption in the gastrointestinal (GI) tract and has a low risk of side effects. Data suggest that ezetimibe will lower total cholesterol by 20-30 mg/dL. *HMG-CoA reductase inhibitors (statins) are the drug of choice for treatment of FH because of their remarkable effectiveness and acceptable risk profile.* This class of drugs in children over age 10 years is as effective in children as in adults, and the risks of elevated hepatic enzymes and myositis are no greater than in adults. Another class of drugs, the proprotein convertase subtilisin/kexin type 9 (PCSK-9) inhibitors, are monoclonal antibodies (mAbs) that block the action of PCSK-9 to downregulate the LDL-R. These agents boost LDL-R levels and result in a marked decrease in plasma LDL-C levels. PCSK-9 inhibitors have a role in adults intolerant of statins and those with subtherapeutic statin effect. *Use of evolocumab in children over age 10 is FDA approved.* In addition, tobacco avoidance and cessation of use of tobacco in the family should be stressed from the time of diagnosis.

Familial Defective ApoB-100

Familial defective apoB-100 is an autosomal dominant condition indistinguishable from heterozygous *LDLR* FH. LDL cholesterol levels are increased, triglycerides are normal, adults often develop tendon xanthomas, and premature CHD occurs. Familial defective apoB-100 is caused by a pathogenic variant in the receptor-binding region of apoB-100, the ligand of the LDL receptor, with an estimated frequency of 1 in 700 people in Western cultures. It is usually caused by missense substitution of apoB-100 (p.Arg3527Gln, previously numbered p.Arg3500Gln), which results in reduced ability of the LDL-R to bind LDL-C, thus impairing its removal from the circulation. Specialized laboratory testing can distinguish familial defective apoB-100 from FH, but this is not necessary, except in research settings, because treatment is the same.

Autosomal Recessive Hypercholesterolemia

This rare condition, caused by a defect in LDL-R–mediated endocytosis in the liver, clinically presents with severe hypercholesterolemia at levels intermediate between those found in homozygous and heterozygous FH. It is disproportionately present among Sardinians, reported in other Mediterranean populations, and is modestly responsive to treatment with HMG-CoA reductase inhibitors.

Sitosterolemia

A rare autosomal recessive condition characterized by excessive intestinal adsorption of plant sterols, sitosterolemia is caused by pathogenic variants in the adenosine triphosphate (ATP)–binding cassette transporter system (*ABCG5* or *ABCG8*), which is responsible for limiting adsorption of plant sterols in the small intestine and promotes biliary excretion of the small amounts adsorbed. Plasma cholesterol levels may be severely elevated, resulting in tendon xanthomas and premature atherosclerosis. Other features include hemolytic anemia, macrothrombocytopenia (large platelets, reduced number), and hemorrhage. Diagnosis can be confirmed by measuring elevated plasma sitosterol levels. Treatment with HMG-CoA reductase inhibitors is not effective, but cholesterol adsorption inhibitors, such as ezetimibe, and bile acid sequestrants are effective.

Polygenic Hypercholesterolemia

Primary elevation in LDL-C among children and adults is most often polygenic; the small effects of many genes are affected by environmental influences (diet). Plasma cholesterol levels are modestly elevated; triglyceride levels are normal. Polygenic hypercholesterolemia aggregates in families sharing a common lifestyle but does not follow predictable hereditary patterns found in single-gene lipoprotein defects. Treatment of children with polygenic hypercholesterolemia is directed

Table 106.8 Percentage of Youths Younger than Age 18 years Expected to Have Familial Hypercholesterolemia (FH) According to Cholesterol Levels and Closest Relative with FH

TOTAL CHOL (mg/dL)	LDL CHOL (mg/dL)	PERCENTAGE WITH FH AT THAT LEVEL			
		DEGREE OF RELATIVE			
		FIRST	SECOND	THIRD	GENERAL POPULATION
180	122	7.2	2.4	0.9	0.01
190	130	13.5	5.0	2.2	0.03
200	138	26.4	10.7	4.9	0.07
210	147	48.1	23.6	11.7	0.19
220	155	73.1	47.5	27.9	0.54
230	164	90.0	75.0	56.2	1.8
240	172	97.1	93.7	82.8	6.3
250	181	99.3	97.6	95.3	22.2
260	190	99.9	99.5	99.0	57.6
270	200	100.0	99.9	99.8	88.0
280	210	100.0	100.0	100.0	97.8
290	220	100.0	100.0	100.0	99.6
300	230	100.0	100.0	100.0	99.9
310	240	100.0	100.0	100.0	100.0

Chol, Cholesterol; LDL, low-density lipoprotein.
From Williams RR, Hunt SC, Schumacher MC, et al. Diagnosing heterozygous familial hypercholesterolemia using new practical criteria validated by molecular genetics. *Am J Cardiol.* 1993;72:171–176.

toward adoption of a healthy lifestyle: reduced total and saturated fat consumption and at least 1 hour of physical activity daily. Cholesterol-lowering medication is rarely necessary.

Hypercholesterolemia with Hypertriglyceridemia

Familial Combined Hyperlipidemia

This autosomal dominant condition is characterized by moderate elevation in plasma LDL-C and triglycerides and reduced plasma HDL-C. Familial combined hyperlipidemia (FCHL) is the most common primary lipid disorder, affecting approximately 1 in 200 people. Family history of premature heart disease is typically positive; the formal diagnosis requires that at least two first-degree relatives have evidence of one of three variants of dyslipidemia: (1) >90th percentile plasma LDL-C, (2) >90th percentile LDL-C and triglycerides, and (3) >90th percentile triglycerides. Individuals can switch from one phenotype to another. Xanthomas are not a feature of FCHL. Elevated plasma apoB levels with increased small, dense LDL particles support the diagnosis.

Children and adults with FCHL have coexisting adiposity, hypertension, and hyperinsulinemia, suggesting the presence of **metabolic syndrome**. Formal diagnosis in adults, as defined by the National Cholesterol Education Program (NCEP) Adult Treatment Panel III, identifies six major components: abdominal obesity, atherogenic dyslipidemia, hypertension, insulin resistance with or without impaired glucose tolerance, evidence of vascular inflammation, and prothrombotic state. An estimated 30% of overweight adults fulfill criteria for the diagnosis of metabolic syndrome, including 65% of those with FCHL. *There is no official definition of metabolic syndrome for children.* Absolute cutoffs for diagnosis in children do not account for continuous variables in aging, sexual maturation, and race/ethnicity.

FCHL and type 2 diabetes share many features of metabolic syndrome, suggesting that they are less distinct entities than originally conceptualized. Genetic association studies reveal evidence for a common genetic background. The resultant metabolic overlap is associated with ectopic fat accumulation and insulin resistance. The mechanisms associating visceral adiposity with metabolic syndrome and type 2 diabetes are not fully understood. It is assumed that hypercholesterolemia and, with less certainty, hypertriglyceridemia confer risk for CVD in patients with FCHL. When features of metabolic syndrome are included in logistic models, shared etiologic features such as increased visceral adiposity become apparent. Visceral adiposity increases with age, and its importance in children as a risk factor for heart disease and diabetes is limited by the relative paucity of data. Body mass index (BMI) remains the surrogate for adiposity in the pediatric clinical setting.

The cornerstone of management is lifestyle modification. This includes a diet low in saturated fats, *trans* fats, and cholesterol, as well as reduced consumption of processed sugars. Increased dietary intake of fruits and vegetables is important, as is 1 hour of moderate physical activity daily. Compliance among children and their parents is often a problem, but small incremental steps are more likely to succeed than aggressive weight loss strategies. It is very important that the child's caregivers participate in the process. Plasma triglyceride levels are usually quite responsive to dietary restriction, especially reduction in the amount of sweetened drinks consumed. Blood cholesterol levels may decrease by 10–15%, but if LDL-C remains >160 mg/dL, drug therapy should be considered.

Familial Dysbetalipoproteinemia (Type III Hyperlipoproteinemia)

Familial dysbetalipoproteinemia (FDBL) is caused by pathogenic variants in *APOE,* which, when exposed to environmental influences (e.g., high-fat high-caloric diet, excessive alcohol intake), results in a mixed type of hyperlipidemia. Patients tend to have elevated plasma cholesterol and triglycerides to a relatively similar degree. HDL-C is typically normal, in contrast to other causes of hypertriglyceridemia associated with low HDL. This rare disorder affects approximately 1 in 10,000 persons. ApoE mediates removal of chylomicron and VLDL remnants from the circulation by binding to hepatic surface receptors. The polymorphic *APOE* gene expresses in three isoforms: *apoE3, apoE2,* and *apoE4. E4* is the "normal" allele present in the majority of the population. The *apoE2* isoform has lower affinity for the LDL receptor, and its frequency is approximately 7%. Approximately 1% of the population is

Table 106.9 Plasma Cholesterol and Triglyceride Levels in Childhood and Adolescence: Means and Percentiles

	TOTAL TRIGLYCERIDE (mg/dL)					TOTAL CHOLESTEROL (mg/dL)					LDL CHOLESTEROL (mg/dL)					HDL CHOLESTEROL (mg/dL)*				
	5TH	MEAN	75TH	90TH	95TH	5TH	MEAN	75TH	90TH	95TH	5TH	MEAN	75TH	90TH	95TH	5TH	10TH	25TH	MEAN	95TH
Cord	14	34	—	—	84	42	68	—	—	103	17	29	—	—	50	13	—	—	35	60
1-4 YR																				
Male	29	56	68	85	99	114	155	170	190	203	—	—	—	—	—	—	—	—	—	—
Female	34	64	74	95	112	112	156	173	188	200	—	—	—	—	—	—	—	—	—	—
5-9 YR																				
Male	28	52	58	70	85	125	155	168	183	189	63	93	103	117	129	38	42	49	56	74
Female	32	64	74	103	126	131	164	176	190	197	68	100	115	125	140	36	38	47	53	73
10-14 YR																				
Male	33	63	74	94	111	124	160	173	188	202	64	97	109	122	132	37	40	46	55	74
Female	39	72	85	104	120	125	160	171	191	205	68	97	110	126	136	37	40	45	52	70
15-19 YR																				
Male	38	78	88	125	143	118	153	168	183	191	62	94	109	123	130	30	34	39	46	63
Female	36	73	85	112	126	118	159	176	198	207	59	96	111	29	137	35	38	43	52	74

*Note that different percentiles are listed for high-density lipoprotein (HDL) cholesterol.
LDL, Low-density lipoprotein.
Data for cord blood from Strong W. Atherosclerosis: its pediatric roots. In Kaplan N, Stamler J, eds. *Prevention of Coronary Heart Disease*. Philadelphia: Saunders; 1983. Data for children 1-4 yr from Tables 6, 7, 20, and 21, and all other data from Tables 24, 25, 32, 33, 36, and 37 in *Lipid Research Clinics Population Studies Data Book*, Vol 1, "The Prevalence Study," NIH Publication No. 80-1527. Washington, DC: National Institutes of Health; 1980

homozygous for *apoE2/E2,* the most common pathogenic variant associated with FDBL, but only a minority expresses the disease. Expression requires precipitating illnesses such as diabetes, obesity, renal disease, or hypothyroidism. Individuals homozygous for *apoE4/E4* are at risk for late-onset Alzheimer disease and dementia from repeated sports-related head injuries.

Most patients with FDBL present in adulthood with distinctive xanthomas. Tuberoeruptive xanthomas resemble small, grapelike clusters on the knees, buttocks, and elbows. Prominent orange-yellow discoloration of the creases of the hands (palmar xanthomas) is also typically present. Atherosclerosis, often presenting with peripheral vascular disease, usually occurs in the fourth or fifth decade. Children may present with a less distinctive rash and generally have precipitating illnesses.

The diagnosis of FDBL is established by lipoprotein electrophoresis, which demonstrates a broad beta band containing remnant lipoproteins. Direct measurement of VLDL by ultracentrifugation can be performed in specialized lipid laboratories. A VLDL/total triglyceride ratio >0.30 supports the diagnosis. *APOE* genotyping for *apoE2* homozygosity can be performed, confirming the diagnosis in the presence of the distinctive physical findings. A negative result does not necessarily rule out the disease, as other pathogenic variants in *APOE* may cause even more serious manifestations.

Pharmacologic treatment of FDBL is necessary to decrease the likelihood of symptomatic atherosclerosis in adults. HMG-CoA reductase inhibitors, nicotinic acid, and fibrates are all effective. FDBL is quite responsive to recommended dietary restriction.

Hypertriglyceridemias

The familial disorders of triglyceride-rich lipoproteins include both common and rare variants of the Frederickson classification system. These include familial chylomicronemia (type I), familial hypertriglyceridemia (type IV), and the more severe combined hypertriglyceridemia and chylomicronemia (type V). Hepatic lipase deficiency also results in a similar combined hyperlipidemia.

Familial Chylomicronemia (Type I Hyperlipidemia)

This rare single-gene defect, like FH, is caused by pathogenic variants affecting clearance of apoB-containing lipoproteins. A deficiency or absence of lipoprotein lipase *(LPL)* or its cofactor apoC-II *(APOC2)*, which facilitates lipolysis by LPL, causes severe elevation of triglyceride-rich plasma chylomicrons. HDL-C levels are decreased. Clearance of these particles is greatly delayed, so the plasma is noted to have a turbid appearance even after prolonged fasting (Fig. 106.13). Chylomicronemia caused by LPL deficiency is associated with modest elevation in triglycerides, whereas this is not the case when the cause is deficient or absent apoC-II. Both are autosomal recessive conditions with a frequency of approximately 1 in 1 million. The disease usually presents during childhood with acute pancreatitis. Eruptive xanthomas on the arms, knees, and buttocks may be present, and there may be hepatosplenomegaly. The diagnosis is established by assaying triglyceride lipolytic activity. **Treatment** of chylomicronemia is by vigorous dietary fat restriction supplemented by fat-soluble vitamins. Medium-chain triglycerides that are adsorbed into the portal venous system may augment total fat intake, and administration of fish oils may also be beneficial.

Familial Hypertriglyceridemia (Type IV Hyperlipidemia)

Familial hypertriglyceridemia (FHTG) is an autosomal dominant disorder of unknown etiology that occurs in approximately 1 in 500 individuals. It is characterized by elevation of plasma triglycerides >90th percentile (250-1,000 mg/dL range), often accompanied by slight elevation in plasma cholesterol and low HDL. FHTG does not usually manifest until adulthood, although it can be detected in approximately 20% of affected children. In contrast to FCHL, FHTG is not thought to be highly atherogenic. It is most likely caused by defective breakdown of VLDL or, less often, by overproduction of this class of lipoproteins.

The diagnosis should include the presence of at least one first-degree relative with hypertriglyceridemia. FHTG should be distinguished from FCHL and FDBL, which require more vigorous treatment to prevent coronary or peripheral vascular disease. The differentiation is usually possible on clinical grounds, in that lower LDL-C levels accompany FHTG, but measurement of normal apoB levels in FHTG may be helpful in ambiguous situations.

Fig. 106.13 Milky plasma from a patient with acute abdominal pain. *(From Durrington P. Dyslipidaemia, Lancet. 2003;362:717–731.)*

A more severe hypertriglyceridemia characterized by increased levels of chylomicrons and VLDL particles (Frederickson **type V**) may occasionally be encountered. Triglyceride levels are often >1,000 mg/dL. The disease is rarely seen in children. In contrast to chylomicronemia (Frederickson **type I**), LPL or apoC-II deficiency is not present. These patients often develop eruptive xanthomas in adulthood, whereas **type IV** hypertriglyceridemia individuals do not. Acute pancreatitis may be the presenting illness. As with other hypertriglyceridemias, excessive alcohol consumption and estrogen therapy can exacerbate the disease.

Secondary causes of *transient hypertriglyceridemia* should be ruled out before making a diagnosis of FHTG. A diet high in simple sugars and carbohydrates or excessive alcohol consumption, as well as estrogen therapy, may exacerbate hypertriglyceridemia. Adolescents and adults should be questioned about excessive consumption of soda and other sweetened drinks, as it is common to encounter people who drink supersized drinks or multiple 12 oz cans of sweetened drinks daily. Cessation of this practice often results in a dramatic fall in triglyceride levels and weight among those who are obese. HDL-C levels will tend to rise as BMI stabilizes.

Pediatric diseases associated with hyperlipidemia include hypothyroidism, nephrotic syndrome, biliary atresia, glycogen storage disease, Niemann-Pick disease (NPD), Tay-Sachs disease, systemic lupus erythematosus, hepatitis, and anorexia nervosa (Table 106.10). Certain medications exacerbate hyperlipidemia, including isotretinoin (Accutane), thiazide diuretics, second-generation antipsychotic agents, oral contraceptives, corticosteroids, β blockers, immunosuppressants, and protease inhibitors used in HIV treatment.

Treatment of hypertriglyceridemia in children rarely requires medication unless levels >1,000 mg/dL persist after dietary restriction of fats,

Table 106.10 Secondary Causes of Hyperlipidemia

HYPERCHOLESTEROLEMIA
Hypothyroidism
Nephrotic syndrome
Cholestasis
Anorexia nervosa
Drugs: progesterone, thiazides, carbamazepine (Tegretol), cyclosporine
HYPERTRIGLYCERIDEMIA
Glycogen storage disease type 1
Obesity
Type 2 diabetes
Alcohol
Renal failure
Sepsis
Stress
Cushing syndrome
Pregnancy
Hepatitis
AIDS, protease inhibitors
Drugs: anabolic steroids, β blockers, estrogen, thiazides, Accutane
REDUCED HIGH-DENSITY LIPOPROTEIN
Smoking
Obesity
Type 2 diabetes
Malnutrition
Drugs: β blockers, anabolic steroids

sugars, and carbohydrates, accompanied by increased physical activity. In such patients the aim is to prevent episodes of pancreatitis. The common use of fibrates (fenofibric acid) and niacin in adults with hypertriglyceridemia is not recommended in children. HMG-CoA reductase inhibitors are variably effective in lowering triglyceride levels, and there is considerably more experience documenting the safety and efficacy of this class of lipid-lowering medications in children. In adults, the U.S. Food and Drug Administration (FDA) has approved prescription (Lovaza, Vascepa) and nonprescription fish oils as adjuncts to diet in the treatment of severe hypertriglyceridemias.

Hepatic Lipase Deficiency

Hepatic lipase deficiency is a very rare autosomal recessive condition caused by pathogenic variants in *LMF1* resulting in elevation in both plasma cholesterol and triglycerides. Hepatic lipase hydrolyzes triglycerides and phospholipids in VLDL remnants and IDL, preventing their conversion to LDL. HDL-C levels tend to be increased rather than decreased, suggesting the diagnosis. Laboratory confirmation is established by measuring hepatic lipase activity in heparinized plasma.

Disorders of High-Density Lipoprotein Metabolism

Primary Hypoalphalipoproteinemia

Isolated low HDL-C is a familial condition that often follows a pattern suggestive of autosomal dominant inheritance but may occur independent of family history. It is the most common disorder of HDL metabolism. It is defined as HDL-C <10th percentile for gender and age with normal plasma triglycerides and LDL-C. Whether it is associated with more rapid atherosclerosis is uncertain. Primary hypoalphalipoproteinemia appears to be related to a reduction in apoA-I synthesis and increased catabolism of HDL. Secondary causes of low HDL-C, such as metabolic syndrome, and rare diseases such as LCAT deficiency and Tangier disease must be ruled out.

Familial Hyperalphalipoproteinemia

This is an unusual condition conferring decreased risk for CHD among family members. Plasma levels of HDL-C exceed 80 mg/dL.

Familial Apolipoprotein A-I Deficiency

Pathogenic variants in the *APOA1* gene may result in complete absence of plasma HDL. Nascent HDL is produced in the liver and small intestine. Free cholesterol from peripheral cells is esterified by LCAT, enabling formation of mature HDL particles. ApoA-I is required for normal enzymatic functioning of LCAT. The resultant accumulation of free cholesterol in the circulation eventually leads to corneal opacities, planar xanthomas, and premature atherosclerosis. Some patients, however, may have pathogenic variants of *APOA1* that result in very rapid catabolism of the protein not associated with atherogenesis, despite HDL-C levels in the 15-30 mg/dL range.

Tangier Disease

This autosomal dominant disease is associated with HDL-C levels <5 mg/dL. It is caused by pathogenic variants in *ABCA1,* which encodes a protein that facilitates the binding of cellular cholesterol to apoA-I. This results in free cholesterol accumulation in the reticuloendothelial system, manifested by tonsillar hypertrophy of a distinctive orange color and hepatosplenomegaly. Intermittent peripheral neuropathy may occur from cholesterol accumulation in Schwann cells. Diagnosis should be suspected in children with enlarged orange tonsils and extremely low HDL-C levels.

Familial Lecithin–Cholesterol Acyltransferase Deficiency

Pathogenic variants of *LCAT* interfere with the esterification of cholesterol, thereby preventing formation of mature HDL particles. This is associated with rapid catabolism of apoA-I. Free circulating cholesterol in the plasma is greatly increased, which leads to corneal opacities and HDL-C levels <10 mg/dL. Partial LCAT deficiency is known as "fish-eye" disease because of the corneal opacities. Complete deficiency causes hemolytic anemia and progressive renal insufficiency early in adulthood. This rare disease is not thought to cause premature atherosclerosis. Laboratory confirmation is based on a demonstration of decreased cholesterol esterification in the plasma.

Cholesteryl Ester Transfer Protein Deficiency

Pathogenic variants of the *CETP* gene result in cholesteryl ester transfer protein (CETP) deficiency. CETP facilitates the transfer of lipoproteins from mature HDL to and from VLDL and chylomicron particles, thus ultimately regulating the rate of cholesterol transport to the liver for excretion in the bile. About half of mature HDL-2 particles are directly removed from the circulation by HDL receptors on the surface of the liver. The other half of cholesteryl esters in the core of HDL exchange with triglycerides in the core of apoB lipoproteins (VLDL, IDL, LDL) for transport to the liver. Homozygous deficiency of CETP has been observed in subsets of the Japanese population with extremely high HDL-C levels (>150 mg/dL).

Conditions Associated with Low Cholesterol

Disorders of apoB-containing lipoproteins and intracellular cholesterol metabolism are associated with low plasma cholesterol.

Abetalipoproteinemia

This rare autosomal recessive disease is caused by pathogenic variants in the *MTTP* gene that encodes the microsomal triglyceride transfer protein necessary for the transfer of lipids to nascent chylomicrons in the small intestine and VLDL in the liver. This results in an absence of chylomicrons, VLDL, LDL, and apoB and very low levels of plasma cholesterol and triglycerides. Fat and fat-soluble vitamin malabsorption, diarrhea, and failure to thrive present in early childhood. Spinocerebellar degeneration, secondary to vitamin E deficiency, manifests in loss of deep tendon reflexes progressing to ataxia and lower-extremity spasticity by adulthood. Patients with abetalipoproteinemia also acquire a progressive pigmented retinopathy associated with decreased night and color vision and eventual blindness. The neurologic symptoms and retinopathy may be mistaken for Friedreich ataxia. Differentiation from Friedreich ataxia is suggested by the presence of malabsorption and acanthocytosis on peripheral blood smear

in abetalipoproteinemia. Many of the clinical manifestations of the disease are a result of malabsorption of fat-soluble vitamins, such as vitamins E, A, and K. *Early treatment with supplemental vitamins, especially E, may significantly slow the development of neurologic sequelae.* Vitamin E is normally transported from the small intestine to the liver by chylomicrons, where it is dependent on the endogenous VLDL pathway for delivery into the circulation and peripheral tissues. Parents of children with abetalipoproteinemia have normal blood lipid and apoB levels.

Familial Hypobetalipoproteinemia

Familial homozygous hypobetalipoproteinemia is associated with symptoms very similar to those of abetalipoproteinemia, but the inheritance pattern is autosomal dominant caused by pathogenic variants in *APOB*. It is distinguishable from abetalipoproteinemia in that heterozygous parents of probands have plasma LDL-C and apoB levels less than half of normal. However, few symptoms or sequelae are associated with the heterozygous condition.

The selective inability to secrete apoB-48 from the small intestine results in a condition resembling abetalipoproteinemia or homozygous hypobetalipoproteinemia. Sometimes referred to as **Anderson disease** or chylomicron retention disease, caused by recessive alterations of the *SAR1B* gene, the failure of chylomicron absorption causes steatorrhea and fat-soluble vitamin deficiency. The blood level of apoB-100, derived from normal hepatocyte secretion, is normal in this condition.

Smith-Lemli-Opitz Syndrome

Smith-Lemli-Opitz syndrome **(SLOS)** is an autosomal recessive disorder that includes microcephaly, polydactyly, holoprosencephaly, seizures and developmental delay. Phenotypic variance ranges from microcephaly, cardiac and brain malformation, and multiorgan system failure to only subtle dysmorphic features and mild developmental delay. It is associated with low plasma cholesterol and accumulated precursors (Tables 106.11 and 106.12) (see Chapter 628.2). Pathogenic variants in the *DHCR7* (7-dehydrocholesterol-Δ7 reductase) gene result in deficiency of the microsomal enzyme DHCR7, which is necessary to complete the final step in cholesterol synthesis. Hypotheses to connect the role of cholesterol synthesis in SLOS congenital malformations have implicated the ligand sonic hedgehog (SHH), for which developmental signaling activity is dependent on cholesterol modification. The incidence of SLOS is estimated to be 1 in 20,000-60,000 births.

Spontaneous abortion of SLOS fetuses may occur. **Type II** SLOS often leads to death by the end of the neonatal period. Survival is unlikely when the plasma cholesterol level is <20 mg/dL. Laboratory measurement should be performed by gas chromatography, because standard techniques for lipoprotein assay include measurement of cholesterol precursors, which may yield a false-positive result. Milder cases may not present until late childhood. **Treatment** includes supplemental dietary cholesterol (egg yolk) and HMG-CoA reductase inhibition to prevent the synthesis of toxic precursors proximal to the enzymatic block.

Disorders of Intracellular Cholesterol Metabolism

Cerebrotendinous Xanthomatosis

This autosomal recessive disorder presents in late adolescence with tendon xanthomas, cataracts, and progressive neurodegeneration. It is caused by tissue accumulation of bile acid intermediates that are shunted into production of cholestanol that in turn result from pathogenic variants in the gene for sterol 27-hydroxylase *(CYP27A1)*. This enzyme is necessary for normal mitochondrial synthesis of bile acids in the liver. Early treatment with chenodeoxycholic acid reduces cholesterol levels and prevents the development of symptoms.

Wolman Disease and Cholesterol Ester Storage Disease

These autosomal recessive disorders are caused by pathogenic variants in *LIPA* that result in lack of lysosomal acid lipase. After LDL cholesterol is incorporated into the cell by endocytosis, it is delivered to lysosomes, where it is hydrolyzed by lysosomal lipase. Failure of hydrolysis because of complete absence of the enzyme causes accumulation of cholesteryl esters within the cells. Hepatosplenomegaly, steatorrhea, and failure to thrive occur during early infancy, leading to death by the end of the first year. In cholesterol ester storage disease, a less severe form than Wolman disease, there is low but detectable acid lipase activity (see Chapter 106.4).

Table 106.11 Major Clinical Characteristics of Smith-Lemli-Opitz Syndrome: Frequent Anomalies (>50% of Patients)

CRANIOFACIAL
Microcephaly
Blepharoptosis
Anteverted nares
Retromicrognathia
Low-set, posteriorly rotated ears
Midline cleft palate
Broad maxillary alveolar ridges
Cataracts (<50%)
SKELETAL ANOMALIES
Syndactyly of toes II/III
Postaxial polydactyly (<50%)
Equinovarus deformity (<50%)
GENITAL ANOMALIES
Hypospadias
Cryptorchidism
Sexual ambiguity (<50%) or XY sex reversal
DEVELOPMENT
Prenatal and postnatal growth retardation
Feeding problems
Intellectual disability
Behavioral abnormalities

From Haas D, Kelley RI, Hoffmann GF. Inherited disorders of cholesterol biosynthesis. *Neuropediatrics.* 2001;32:113–122.

Niemann-Pick Disease Type C

This disorder of intracellular cholesterol transport is characterized by accumulation of cholesterol and sphingomyelin in the CNS and reticuloendothelial system. Death from this autosomal recessive neurologic disease usually occurs by adolescence (see Chapter 106.4).

Lipoprotein Patterns in Children and Adolescents

Derived primarily from the Lipid Research Clinics Population Studies, Table 106.9 shows the distribution of lipoprotein levels in American youth at various ages. Total plasma cholesterol rises rapidly from a mean of 68 mg/dL at birth to a level approximately twice that by the end of the neonatal period. A very gradual rise in total cholesterol level occurs until puberty, when the mean level reaches 160 mg/dL. Total cholesterol falls transiently during puberty in males because of a small decrease in HDL-C and in females secondary to a slight fall in LDL-C. Blood cholesterol levels track reasonably well as individuals age.

High blood cholesterol tends to aggregate in families, a reflection of genetic and environmental influences.

Acceptable total cholesterol among children and adolescents is <170 mg/dL; borderline is 170-199 mg/dL; and high is >200 mg/dL. Acceptable LDL-C is <110 mg/dL; borderline is 110-129 mg/dL; and high is >130 mg/dL. HDL-C should be >40 mg/dL.

Blood Cholesterol Screening

A lipid profile should be checked for all children between ages 9 and 11 years and then another between ages 17 and 21 years because cholesterol levels may vary after puberty. However, if a child would have met the selective criteria from the previous risk-based guidelines (premature coronary artery disease [CAD] in a parent or grandparent, a parent with cholesterol >240 mg/dL), screening can occur as early as age 2

Table 106.12 Characteristic Malformations of Internal Organs in Severely Affected Smith-Lemli-Opitz Patients

CENTRAL NERVOUS SYSTEM
Frontal lobe hypoplasia
Enlarged ventricles
Agenesis of corpus callosum
Cerebellar hypoplasia
Holoprosencephaly
CARDIOVASCULAR
Atrioventricular canal
Secundum atrial septal defect
Patent ductus arteriosus
Membranous ventricular septal defect
URINARY TRACT
Renal hypoplasia or aplasia
Renal cortical cysts
Hydronephrosis
Ureteral duplication
GASTROINTESTINAL
Hirschsprung disease
Pyloric stenosis
Refractory dysmotility
Cholestatic and noncholestatic progressive liver disease
PULMONARY
Pulmonary hypoplasia
Abnormal lobation
ENDOCRINE
Adrenal insufficiency

From Haas D, Kelley RI, Hoffmann GF. Inherited disorders of cholesterol biosynthesis. *Neuropediatrics.* 2001;32:113–122.

years. Data also suggest that obtaining a nonfasting lipid profile can be just as useful in detecting severe genetic dyslipidemias as a fasting lipid profile and thus can be used as first-line screening in children. Fasting lipid profiles may also be used depending on parental, child, and clinician preference, especially if there is concern for hypertriglyceridemia, because triglycerides are affected more by fasting status. Abnormal lipid panels should be repeated, and especially when the concern is the triglycerides, the second panel should be obtained ≥2 weeks later in the fasted state. Treatment other than lifestyle modification is not initiated based on a single lipid panel determination.

Risk Assessment and Treatment of Hyperlipidemia

The NCEP recommends a population-based approach toward a healthy lifestyle applicable to all children and an individualized approach directed at those children at high risk (Fig. 106.14). The important focus on maintenance of a healthy lifestyle rather than aggressive weight reduction is recommended by the American Academy of Pediatrics (AAP).

All children with dyslipidemias are stratified according to the presence of high-level or moderate-level risk factors to determine their ultimate treatment. **High-level risk factors** are defined as hypertension requiring drug therapy (blood pressure ≥99th percentile + 5 mm Hg), current cigarette smoker, BMI at the ≥97th percentile, the presence of type 1 or type 2 diabetes mellitus, chronic kidney disease, postorthoptic heart transplant, and/or Kawasaki disease with current aneurysms. **Moderate-level risk factors** are defined as hypertension that does not require drug therapy, BMI at the ≥95th percentile but <97th percentile, HDL-C <40 mg/dL, Kawasaki disease (and possibly multisystem inflammatory syndrome in children [MIS-C]) with regressed coronary aneurysms, chronic inflammatory disease, HIV infection, and/or the presence of nephrotic syndrome.

The initial treatment for dyslipidemia in a child always begins with a 6-month trial of lifestyle modification, namely, improvements in dietary and physical activity patterns. Being overweight confers a special risk of CVD because of the strong association with insulin resistance syndrome (metabolic syndrome). Although there is no standardized definition of metabolic syndrome for youth, it is likely that half of all severely obese children are insulin resistant. Data from the CARDIAC project noted that 49% of fifth-grade children with the hyperpigmented rash, acanthosis nigricans, had three or more factors for insulin resistance syndrome when using the definition classically used for adults, including evidence of insulin resistance, hypertension, HDL-C <40 mg/dL, and triglycerides >150 mg/dL, in addition to obesity.

The Cardiovascular Health Integrated Lifestyle Diet-1 **(CHILD-1)** diet is the first level of dietary change to be recommended for all children with dyslipidemias. The CHILD-1 diet is specially designed for children with risk factors for CAD and focuses on limiting dietary cholesterol to 300 mg/day, limiting sugary drink consumption, using reduced-fat/skim milk, avoiding foods high in *trans*-type fats, limiting foods high in sodium, and encouraging consumption of foods high in fiber. Specific recommendations depend on the child's age. American Heart Association (AHA) diet guidelines for all persons of all ages recommend these measures plus avoidance of highly processed foods. Specific amounts or percentages of saturated vs monosaturated or polyunsaturated fats are not addressed.

The use of the Cardiovascular Health Integrated Lifestyle Diet-2 **(CHILD-2)** diet is recommended if the CHILD-1 diet alone is unsuccessful. Although similar in many aspects to the CHILD-1 diet, the CHILD-2 diet is geared toward a specific dyslipidemia type; the **CHILD-2 LDL** diet is recommended for children with elevated LDL levels and the **CHILD-2 TG** diet for those presenting with elevated triglycerides. The basic recommendations of calorie consumption for the CHILD-2 diet are as follows: only 25–30% of calories from fat, ≤7% of calories from saturated fat, 10% of calories from monounsaturated fat, and <200 mg/day of cholesterol. If the CHILD-2 LDL diet is recommended, the use of plant sterols and water-soluble fiber is emphasized. If the CHILD-2 TG diet is recommended, increasing consumption of omega-3 fatty acids and complex rather than simple carbohydrates is emphasized.

If followed, these dietary recommendations will provide adequate calories for optimal growth and development without promoting obesity. Compliance on the part of children and their caregivers is challenging. *Children learn eating habits from their parents*. Successful adoption of a healthier lifestyle is much more likely to occur if meals and snacks in the home are applicable to the entire family rather than an individual child. A regular time for meals together as a family is desirable. Grandparents and other nonparental caregivers sometimes need to be reminded not to indulge the child who is on a restricted diet. Additionally, the rise in obesity is prompting some school districts to restrict sweetened drink availability and offer more nutritious cafeteria selections.

Changes in physical activity habits are also an important part of the initial lifestyle modification. The National Association for Sport and Physical Education recommends that children should accumulate at least 60 minutes of age-appropriate physical activity on most days of the week. Extended periods (≥2 hr) of daytime inactivity are discouraged, as is >2 hours of television and other forms of screen time.

Pharmacologic Therapy. See Tables 106.13 and 106.14.

Pharmacologic therapy with cholesterol-lowering medication is the cornerstone of treatment for children who fail to respond to 6 months of rigorous lifestyle modification. It should be considered when one of the following conditions are met (also shown in Fig. 106.14):

- LDL cholesterol remains >190 mg/dL
- LDL cholesterol remains >160 mg/dL with the presence of one high-level risk factor *and/or* at least two moderate-level risk factors
- LDL cholesterol remains >130 mg/dL with the presence of at least two high-level risk factors, one high-level risk factor, *and* at least two moderate-level risk factors, *or* evidence of CAD

HMG-CoA reductase inhibitors, also known as "statins," are remarkably effective in lowering LDL-C levels and reducing plaque

Table 106.13 Drugs Used for the Treatment of Hyperlipidemia

DRUG	MECHANISM OF ACTION	INDICATION	STARTING DOSE
HMG-CoA reductase inhibitors (statins)	↓ Cholesterol and VLDL synthesis ↑ Hepatic LDL receptors	Elevated LDL	5-80 mg every night at bedtime
Bile acid sequestrants: Cholestyramine Colestipol	↑ Bile and excretion	Elevated LDL	 4-32 g daily 5-40 g daily
Nicotinic acid	↓ Hepatic VLDL synthesis	Elevated LDL Elevated TG	100-2,000 mg three times daily
Fibric acid derivatives: Gemfibrozil	↑ LPL ↓ VLDL	Elevated TG	600 mg twice daily
Fish oils	↓ VLDL production	Elevated TG	3-10 g daily
Cholesterol absorption inhibitors:			
Ezetimibe	↓ Intestinal absorption cholesterol	Elevated LDL	10 mg daily
PCSK-9 inhibitor Evolocumab	Upregulation of hepatic LDL receptors to enhance removal of LDL from circulation	Refractory or intolerant to statin	140 mg every 2 weeks or 420 mg subcutaneous injection monthly

LDL, Low-density lipoprotein(s); LPL, lipoprotein lipase; TG, triglycerides; VLDL, very-low-density lipoprotein.

Table 106.14 Adverse Effects of Cholesterol-Lowering Drugs

STATINS
Myalgia, myositis, transaminase elevations, hepatic dysfunction, increased risk of diabetes mellitus
Rare: Rhabdomyolysis, hemorrhagic stroke

EZETIMIBE
Diarrhea, arthralgia, rhabdomyolysis, hepatitis, pancreatitis, thrombocytopenia

PCSK9 INHIBITORS
Nasopharyngitis, upper respiratory tract infection, influenza, back pain, injection site reactions, rash, allergic skin reactions, cognitive effects, antidrug antibodies

BILE ACID SEQUESTRANTS
Constipation, heartburn, nausea, eructation, bloating
Adverse effects are more common with colestipol and cholestyramine and may diminish over time.

FIBRIC ACID DERIVATIVES
Gastrointestinal (GI) disturbances, cholelithiasis, hepatitis, myositis

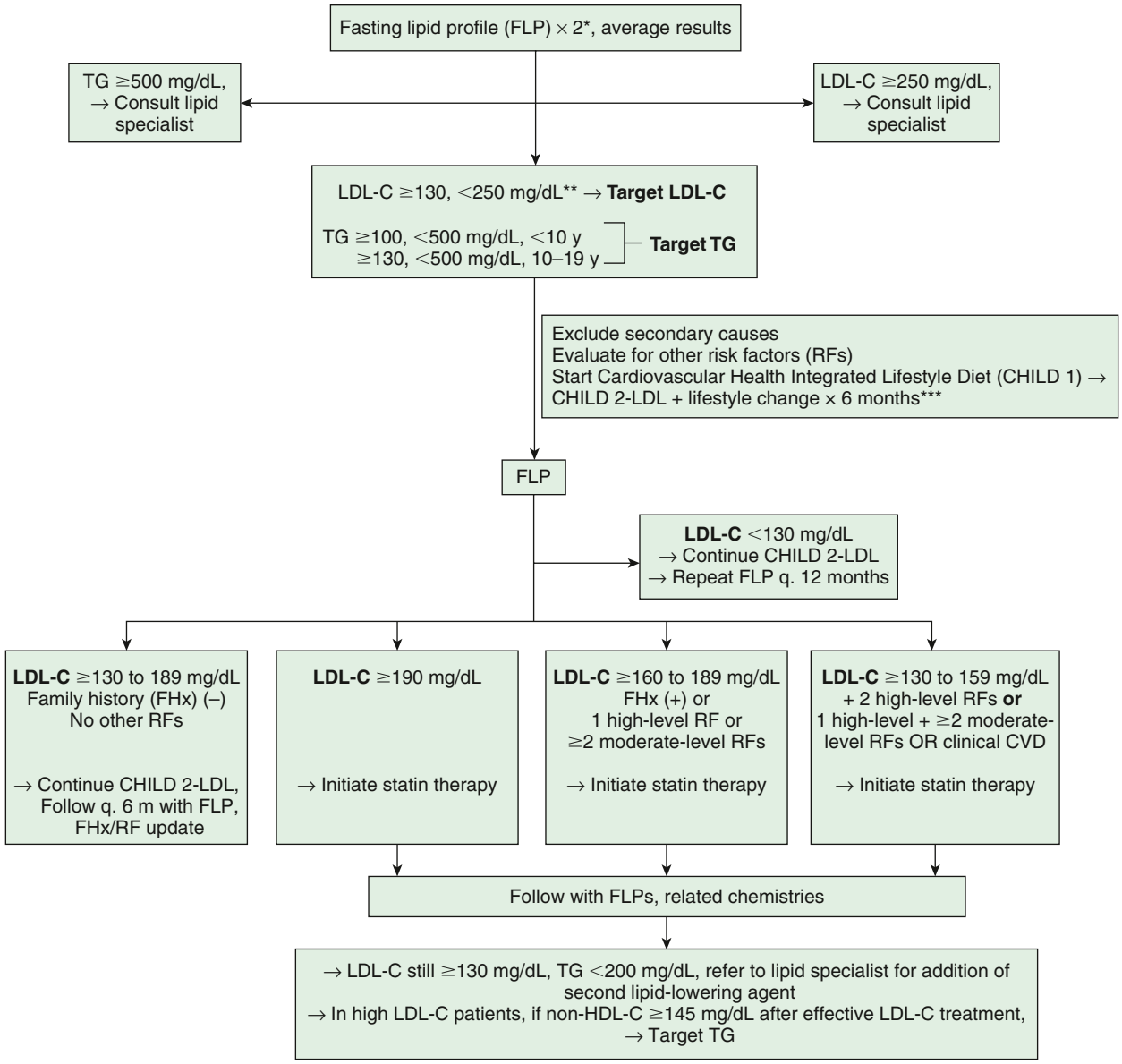
NIACIN
Skin flushing, pruritus, GI disturbances, blurred vision, fatigue, glucose intolerance, hyperuricemia, hepatic toxicity, exacerbation of peptic ulcers
Adverse effects, especially flushing, occur more frequently with immediate-release products.
Rare: Dry eyes, hyperpigmentation

FISH OIL
Eructation, dyspepsia, unpleasant aftertaste

From The Medical Letter. Lipid-lowering drugs. *Med Lett.* 2016;58:133–140, Table 2, p. 136.

inflammation, thereby reducing the likelihood of a sudden coronary event in an at-risk adult within weeks of starting the medication. As a class, they work by blocking the intrahepatic biosynthesis of cholesterol, thereby stimulating the production of more LDL receptors on the cell surface and facilitating the uptake of LDL-C from the bloodstream. The NCEP Adult Treatment Panel advocates aggressive lowering of LDL to <70 mg/dL in individuals with known CAD. This information is relevant because a child who fulfills criteria for consideration of cholesterol-lowering medication will almost always have inherited the condition from one of the child's parents. Not infrequently, when providing care for the child, questions arise about screening and treatment of parents or grandparents. Statins are equally effective in children, capable of lowering LDL-C levels by 50% when necessary. They are considered first-line therapy for children who meet criteria for pharmacologic therapy. They also will effect a modest reduction in triglycerides and an inconsistent increase in HDL-C. Their side effect profile, mainly liver dysfunction and rarely rhabdomyolysis with secondary renal failure, should be taken into consideration before prescribing the drug. However, there has been no evidence that complications are any more frequent in children than in adults, and skeletal muscle discomfort seems to be somewhat less of a problem. Drug interactions may occur as well, so careful attention should be paid to a child's active prescriptions to avoid potentiation of the side effects. Children should have liver enzymes monitored regularly and creatine phosphokinase measured if muscle aches or weakness occurs. Liver (muscle) enzymes may be allowed to rise threefold before discontinuing the drug. There is a suggested link between the use of statins and increased risk of developing type 2 diabetes mellitus in adults, but these results have not been replicated in children. Sex hormones have been measured in children receiving statins and are unchanged. It should be reemphasized that children with modest elevations in cholesterol, such as that seen in polygenic hypercholesterolemia, are not, as a rule, candidates for statins because of their side effect profile and the childhood response to lifestyle modifications. Statins should be started at the lowest effective dose and allowed at least 8 weeks to achieve their peak effect. If LDL levels are not at goal, which in children who are treated is generally established to be <130 mg, the medication may be titrated upward with careful monitoring of side effects.

Other cholesterol-lowering medications, such as nicotinic acid and fibrates, have been used far less often in children than bile acid sequestrants and statins. Nicotinic acid and fibrates have been used selectively in children with marked hypertriglyceridemia (>500 mg/dL) at risk for acute pancreatitis, though dietary restriction of complex sugars (stressing elimination of sugar-sweetened beverages) and carbohydrates will usually result in significant lowering of triglyceride levels. Guidelines recommend treatment of LDL-C as the initial priority, and after LDL levels are at goal, then if triglycerides remain between 200 and 499 mg/dL and non-HDL cholesterol ≥145 mg/dL, pharmacologic treatment to reduce triglyceride levels is indicated. Omega-3 fatty acid supplementation, available in both over-the-counter and prescription form, is a safe and useful treatment thought to reduce triglyceride levels by decreasing the hepatic synthesis of triglycerides. LDL-C levels in adults



* Obtain FLPs at least 2 weeks but no more than 3 months apart.
** Use of drug therapy is limited to children ≥10 y with defined risk profiles.
*** In a child with LDL-C >190 mg/dL and other RFs, trial of CHILD 2-LDL may be abbreviated.

Fig. 106.14 Dyslipidemia treatment algorithm: Target LDL-C (low-density lipoprotein cholesterol). Note: Values given are in mg/dL. To convert to SI units, divide results for total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and non–HDL-C by 38.6; for triglycerides (TG), divide by 88.6. *(From U.S. Department of Health and Human Services, National Institutes of Health, National Heart, Lung, and Blood Institute. Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents. NIH Publication No. 12-7486A, Oct. 2012, Fig 9-1.)*

of about 70 mg/dL were recently associated with coronary artery atheromatous plaque reduction and reversal of CAD. Knowledge in this area will continue to evolve.

Ezetimibe has proved to be useful in the pediatric population because of its efficacy and low side effect profile. Ezetimibe reduces plasma LDL-C by blocking sterol absorption in enterocytes. The drug is marketed as an adjunct to statins when adults are not achieving sufficient blood lipid lowering with statins alone. Sufficient reports documenting its effectiveness without side effects support recommending ezetimibe instead of a statin when moderate hypercholesterolemia is encountered or apprehension from parents makes using a statin difficult.

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106.4 Lipidoses (Lysosomal Storage Disorders)

Margaret M. McGovern and Robert J. Desnick

The lysosomal lipid storage diseases are diverse disorders each caused by an inherited deficiency of a lysosomal hydrolase leading to the intralysosomal accumulation of the enzyme's particular substrate (Tables 106.15 and 106.16). With the exceptions of Wolman disease and cholesterol ester storage disease (see Chapter 106.3), the lipid substrates share a common structure that includes a ceramide backbone (2-N-acylsphingosine) from which the various sphingolipids are derived by substitution of hexoses, phosphorylcholine, or one or more

sialic acid residues on the terminal hydroxyl group of the ceramide molecule. The pathway of sphingolipid metabolism in nervous tissue (Fig. 106.15) and in visceral organs (Fig. 106.16) is known. Each catabolic step, with the exception of the catabolism of lactosylceramide, has a genetically determined metabolic defect and a resultant disease. Because sphingolipids are essential components of all cell membranes, the inability to degrade these substances and their subsequent accumulation results in the physiologic and morphologic alterations and characteristic clinical manifestations of the lipid storage disorders (see Tables 106.15 and 106.16). Progressive lysosomal accumulation of glycosphingolipids in the CNS leads to neurodegeneration, whereas storage in visceral cells can lead to organomegaly, pulmonary infiltration, skeletal abnormalities, and other manifestations.

Diagnostic assays for the identification of affected individuals has relied on the measurement of the specific enzymatic activity in isolated leukocytes or cultured fibroblasts or lymphoblasts. An approach to differentiating these disorders is noted in Figure 106.17. In addition, next generation sequencing of lysosomal genes as well as metabolomic/protomic approaches have helped determine a specific diagnosis. For most disorders, carrier identification and prenatal diagnosis are available; a specific diagnosis is essential to permit genetic counseling. The characterization of the genes that encode the specific enzymes required for sphingolipid metabolism permit the development of therapeutic options, such as recombinant enzyme replacement therapy, as well as the potential of cell or gene therapy. Identification of specific disease-causing pathogenic variants improve diagnosis, prenatal detection, and carrier identification. For several disorders (Gaucher, Fabry, and NPD), it has been possible to make genotype-phenotype correlations that predict disease severity and allow more precise genetic counseling. Inheritance is autosomal recessive except for X-linked Fabry disease.

GM_1 GANGLIOSIDOSIS

GM_1 gangliosidosis most frequently presents in early infancy but has been described in patients with juvenile- and adult-onset subtypes (Fig. 106.18, Table 106.17). Inherited as an autosomal recessive trait, each subtype results from a different gene pathogenic variant that leads to the deficient activity of β-galactosidase, a lysosomal enzyme encoded by the gene *GLB1*. Although the disorder is characterized by the pathologic accumulation of GM_1 gangliosides in the lysosomes of both neural and visceral cells, GM_1 ganglioside accumulation is most marked in the brain. In addition, keratan sulfate, a mucopolysaccharide, accumulates in liver and is excreted in the urine of patients with GM_1 gangliosidosis.

The clinical manifestations of the infantile form of GM_1 gangliosidosis may be evident in the newborn as hepatosplenomegaly, edema, and skin eruptions (angiokeratoma). It most frequently presents in the first 6 months of life with developmental delay followed by progressive psychomotor retardation and the onset of tonic-clonic seizures. Typical facies is characterized by low-set ears, frontal bossing, a depressed nasal bridge, and an abnormally long philtrum. Up to 50% of patients have a macular cherry red spot. Hepatosplenomegaly and skeletal abnormalities similar to those of the mucopolysaccharidoses (see Chapter 109), including anterior beaking of the vertebrae, enlargement of the sella turcica, and thickening of the calvarium, are present. By the end of the first year of life, most patients are blind and deaf, with severe neurologic impairment characterized by decerebrate rigidity. Death usually occurs by 3-4 years of age. The *juvenile-onset* form of GM_1 gangliosidosis is clinically distinct, with a variable age at onset. Affected patients present primarily with neurologic symptoms including ataxia, dysarthria, intellectual disabilities, and spasticity. Deterioration is slow; patients may survive through the fourth decade of life. These patients lack the visceral involvement, facial abnormalities, and skeletal features seen in type 1 disease. *Adult-onset* patients have been described who present with gait and speech abnormalities, dystonia, and mild skeletal abnormalities. There is no specific treatment for any form of GM_1 gangliosidosis.

The diagnosis of GM_1 gangliosidosis should be suspected in infants with typical clinical features and is confirmed by the demonstration of biallelic pathogenic variants in *GLB1* and, as needed, demonstration of deficiency of β-galactosidase activity in peripheral leukocytes. Other disorders that share some of the features of the GM_1 gangliosidoses include Hurler disease (mucopolysaccharidosis type I), I-cell disease, and NPD type A, each of which can be distinguished by the demonstration of their specific genetic variants and enzymatic deficiencies. Carriers of the disorder are detected by the measurement of the enzymatic activity in peripheral leukocytes or by identifying the specific gene pathogenic variants. Prenatal diagnosis is accomplished by testing of known pathogenic variants or by determination of the enzymatic activity in cultured amniocytes or chorionic villi. Currently only supportive therapy is available for patients with GM_1 gangliosidosis.

THE GM_2 GANGLIOSIDOSES

The GM_2 gangliosidoses include the autosomal recessive Tay-Sachs disease and Sandhoff disease. Each results from the deficiency of β-hexosaminidase activity and the lysosomal accumulation of GM_2 gangliosides, particularly in the CNS. Both disorders have been classified into infantile-, juvenile-, and adult-onset forms based on the age at onset and clinical features. β-Hexosaminidase occurs as two isozymes: β-hexosaminidase A, which is composed of one α and one β subunit, and β-hexosaminidase B, which has two β subunits. β-Hexosaminidase A deficiency results from pathogenic variants in the α subunit and causes Tay-Sachs disease, whereas pathogenic variants in the β-subunit gene result in the deficiency of both β-hexosaminidases A and B and cause Sandhoff disease. Both are autosomal recessive traits, with Tay-Sachs disease having a predilection for the Ashkenazi Jewish population, where the carrier frequency is about 1/25.

More than 100 pathogenic variants in *HEXA* have been identified for Tay-Sachs disease. Most are associated with the infantile forms of disease. Three pathogenic variants account for >98% of mutant alleles among Ashkenazi Jewish carriers, including one allele associated with the adult-onset form. Pathogenic variants that cause the subacute or chronic forms result in enzyme proteins with residual enzymatic activities, the levels of which correlate with the severity of the disease.

Patients with the infantile form of Tay-Sachs disease have clinical manifestations in infancy including loss of motor skills, increased startle reaction, and macular pallor and retinal cherry red spots (see Table 106.15). Affected infants usually develop normally until 4-5 months of age, when decreased eye contact and an exaggerated startle response to noise (hyperacusis) are noted. Macrocephaly, not associated with hydrocephalus, may develop. In the second year of life, seizures develop, which may be refractory to anticonvulsant therapy. Neurodegeneration is relentless, with death occurring by the age of 4 or 5 years. The juvenile- and later-onset forms initially present with ataxia and dysarthria and may not be associated with a macular cherry red spot.

The clinical manifestations of Sandhoff disease are similar to those for Tay-Sachs disease. Infants with Sandhoff disease have hepatosplenomegaly, cardiac involvement, and mild bony abnormalities. The juvenile form of this disorder presents as ataxia, dysarthria, and intellectual deterioration, but without visceral enlargement or a macular cherry red spot. More than 50 pathogenic variants in *HEXB* have been identified for Sandhoff disease.

The diagnosis of infantile Tay-Sachs disease and Sandhoff disease is usually suspected in an infant with neurologic features and a cherry red spot. Definitive diagnosis is made by genetic testing or by determination of enzyme activities in peripheral leukocytes. The two disorders can be distinguished enzymatically, because in Tay-Sachs disease only the β-hexosaminidase A isozyme is deficient, whereas in Sandhoff disease both the β-hexosaminidase A and B isozymes are deficient. At-risk pregnancies for both disorders can be prenatally diagnosed by testing known pathogenic variants or determining enzyme levels in fetal cells obtained by amniocentesis or chorionic villus sampling. Identification of carriers in families is also possible by genetic or enzymatic determination. For Tay-Sachs disease, carrier screening of all couples in which at least one member is of Ashkenazi Jewish descent is recommended before the initiation of pregnancy to identify couples at risk. The incidence of Tay-Sachs disease has been markedly reduced since the introduction of carrier screening programs in the Ashkenazi Jewish population. Newborn screening may be possible by measuring specific glycosphingolipid markers or the relevant enzymatic activities in dried

Table 106.15 Clinical Findings in Lysosomal Storage Diseases

NATURE	ENZYME DEFECT	HYDROPS FETALIS	COARSE FACIAL FEATURES DYSOSTOSIS MULTIPLEX	HEPATOSPLENOMEGALY
MUCOLIPIDOSES				
Mucolipidoses II, I-cell disease	*N*-Acetylglucosaminylphosphotransferase	(+)	++	+
Mucolipidosis III, Pseudo-Hurler	N-Acetylglucosaminylphosphotransferase	–	+	(+)
Mucolipidosis IV	Unknown	–	–	+
SPHINGOLIPIDOSES				
Fabry disease	α-Galactosidase	–	–	–
Farber disease	Ceramidase	–	–	(+)
Galactosialidosis	β-Galactosidase and sialidase	(+)	++	++
GM1 gangliosidosis	β-Galactosidase	(+)	++	+
GM2 gangliosidosis (Tay-Sachs disease, Sandhoff disease)	β-Hexosaminidases A and B	–	–	(+)
Gaucher type I	Glucocerebrosidase	–	–	++
Gaucher type II	Glucocerebrosidase	(+)	–	++
Gaucher type III	Glucocerebrosidase	(+)	–	+
Niemann-Pick type A	Sphingomyelinase	(+)	–	++
Niemann-Pick type B	Sphingomyelinase	–	–	++
Metachromatic leukodystrophy	Arylsulfatase A	–	–	–
Krabbe disease	β-Galactocerebrosidase	–	–	–
LIPID STORAGE DISORDERS				
Niemann-Pick type C	Intracellular cholesterol transport	–	–	(+)
Wolman disease	Acid lipase	(+)	–	+
Ceroid lipofuscinosis, infantile (Santavuori-Hantia)	Palmitoyl-protein thioesterase (CLN1)	–	–	–
Ceroid lipofuscinosis, late infantile (Jansky-Bielschowsky)	Pepstatin-insensitive peptidase (CLN2); variants in Finland (CLN5), Turkey (CLN7), and Italy (CLN6)	–	–	–
Ceroid lipofuscinosis, juvenile (Spielmeyer-Vogt)	CLN3, membrane protein	–	–	–
Ceroid lipofuscinosis, adult (Kufs, Parry)	CLN4, probably heterogeneous	(+)	–	–
OLIGOSACCHARIDOSES				
Aspartylglucosaminuria	Aspartylglucosaminase	–	+	(+)
Fucosidosis	α-Fucosidase	–	++	(+)
α-Mannosidosis	α-Mannosidase	–	++	+
β-Mannosidosis	β-Mannosidase	–	+	(+)
Schindler disease	α-N-Acetylgalactosaminidase	–	–	–
Sialidosis I	Sialidase	(+)	–	–
Sialidosis II	Sialidase	(+)	++	+

++, prominent; +, often present, (+), inconstant or occurring later in the disease course; –, not present.
GAG, glycosaminoglycans.
Modified from Hoffmann GF, Nyhan WL, Zschoke J, et al. *Storage Disorders in Inherited Metabolic Diseases.* Philadelphia: Lippincott Williams & Wilkins; 2002:346–351.

Table 106.15 Clinical Findings in Lysosomal Storage Diseases—cont'd

CARDIAC INVOLVEMENT CARDIAC FAILURE	MENTAL DETERIORATION	MYOCLONUS	SPASTICITY	PERIPHERAL NEUROPATHY	CHERRY RED SPOT	CORNEAL CLOUDING	ANGIOKERATOMATA
++	++	–	–	–	–	(+)	–
–	(+)	–	–	–	–	+	–
–	(+)	–	–	–	–	–	–
+	–	–	–	–	–	+	++
++	+	–	–	+	(+)	–	–
+	++	(+)	+	–	+	+	+
(+)	++	–	(+)	–	(+)	+	+
–	++	+	+	–	++	–	–
–	–	–	–	–	–	–	–
–	++	+	+	–	–	–	–
–	+	(+)	(+)	–	–	–	–
–	+	(+)	–	(+)	(++)	–	–
–	–	–	–	(+)	(+)	–	–
–	++	–	+	++	(+)	–	–
–	++	–	+	++	(+)	–	–
–	+	–	–	–	(+)	–	–
(+)	–	–	–	–	(+)	–	–
–	+	+	+	–	–	–	–
–	+	+	+	–	–	–	–
–	+	–	(+)	–	–	–	–
–	+	–	–	–	–	–	–
(+)	+	–	–	–	–	(+)	(+)
+	++	+	+	–	–	–	(+)
–	++	–	(+)	–	–	++	(+)
–	+	–	+	+	–	–	(+)
–	+	+	+	–	–	–	–
–	–	++	+	+	++	(+)	–
+	++	(+)	–	–	++	–	+

Table 106.16 Lysosomal Storage Disorders in the Newborn Period: Genetic and Clinical Characteristics of Neonatal Presentation

DISORDER	ONSET	FACIES	NEUROLOGIC FINDINGS	DISTINCTIVE FEATURES
Niemann-Pick A disease	Early infancy	Frontal bossing	Difficulty feeding, apathy, deafness, blindness, hypotonia	Brownish-yellow skin, xanthomas
Niemann–Pick C disease	Birth to 3 mo	Normal	Developmental delay, vertical gaze paralysis, hypotonia, later spasticity	–
Gaucher disease type 2	In utero to 6 mo	Normal	Poor suck and swallow, weak cry, squint, trismus, strabismus, opsoclonus, hypertonic, later flaccidity	Congenital ichthyosis, collodion skin
Krabbe disease	3-6 mo	Normal	Irritability, tonic spasms with light or noise stimulation, seizures, hypertonia, later flaccidity	Increased CSF protein level
GM1 gangliosidosis	Birth	Coarse	Poor suck, weak cry, lethargy, exaggerated startle, blindness, hypotonia, later spasticity	Gingival hypertrophy, edema, rashes
Farber disease type I	2 wk to 4 mo	Normal	Progressive psychomotor impairment, seizures, decreased reflexes, hypotonia	Joint swelling with nodules, hoarseness, lung disease, contractures, fever, granulomas, dysphagia, vomiting, increased CSF protein level
Farber disease types II and III	Birth to 9 mo (≤20 mo)	Normal	—	Joint swelling with nodules, hoarseness
Farber disease type IV (neonatal)	Birth	Normal	Nodules not consistent findings	Corneal opacities (1/3)
Congenital sialidosis	In utero to birth	Cognitive, edema	Intellectual impairment, hypotonia	Neonatal ascites, inguinal hernias, renal disease
Galactosialidosis	In utero to birth	Coarse	Intellectual impairment, occasional deafness, hypotonia	Ascites, edema, inguinal hernias, renal disease, telangiectasias
Wolman disease	First weeks of life	Normal	Cognitive deterioration	Vomiting, diarrhea, steatorrhea, abdominal distention, failure to thrive, anemia, adrenal calcifications
Infantile sialic acid storage disease	In utero to birth	Coarse, dysmorphic	Intellectual impairment, hypotonia	Ascites, anemia, diarrhea, failure to thrive
I-cell disease	In utero to birth	Coarse	Intellectual impairment, deafness	Gingival hyperplasia, restricted joint mobility, hernias
Mucolipidosis type IV	Birth to 3 mo	Normal	Intellectual impairment, hypotonia	—
Mucopolysaccharidosis type VII	In utero to childhood	Variable coarseness	Mild to severe intellectual impairment	Hernias

Table 106.16 Lysosomal Storage Disorders in the Newborn Period: Genetic and Clinical Characteristics of Neonatal Presentation—cont'd

EYE FINDINGS	DEFECT	GENE LOCATION/MOLECULAR FINDINGS	ETHNIC PREDILECTION
Cherry-red spot (50%)	Sphingomyelinase deficiency	*SMPD1* gene at 11p15.4; 3 of 18 variants account for approximately 92% of mutant alleles in the Ashkenazi population	1:40,000 in Ashkenazi Jews with a carrier frequency of 1:60
–	Abnormal cholesterol esterification	*NPC1* gene at 18q11 accounts for >95% of cases; *HE1* gene variants may account for remaining cases	Increased in French Canadians of Nova Scotia and Spanish Americans in the Southwest United States
–	Glucocerebrosidase deficiency	1q21; large number of variants known; five variants account for approximately 97% of mutant alleles in the Ashkenazi population but approximately 75% in the non-Jewish population	Panethnic
Optic atrophy	Galactocerebrosidase deficiency	14q 24.3-q32.1; >60 variants with some common variants in specific populations	Increased in Scandinavian countries and in a large Druze kindred in Israel
Cherry red spot (50%)	β-Galactosidase deficiency	3pter-3p21; heterogeneous variants; common variants in specific populations	Panethnic
Grayish opacification surrounding the retina in some patients, subtle cherry red spot	Lysosomal acid ceramidase	8p21.3-22; nine disease-causing variants identified	Panethnic
Normal macula, corneal opacities	—	8p21.3-p22	Panethnic
—	—	Unknown	Panethnic
Corneal clouding	Neuraminidase deficiency	*NEU 1* gene (sialidase) at 6p21	Panethnic
Cherry red spot, corneal clouding	Absence of a protective protein that safeguards neuraminidase and β-galactosidase from premature degradation	20q13.1	Panethnic
—	Lysosomal acid lipase deficiency	10q23.2-q23.3; variety of variants identified	Increased in Iranian Jews and in non-Jewish and Arab populations of Galilee
—	Defective transport of sialic acid out of the lysosome	*SLC17A5* gene at 6q	Panethnic
Corneal clouding	Lysosomal enzymes lack mannose 6-phosphate recognition marker and fail to enter the lysosome (phosphotransferase deficiency, 3-subunit complex [α2 β2 γ2])	Enzyme encoded by two genes; α and β subunits encoded by gene at 12p; γ subunit encoded by gene at 16p	Panethnic
Severe corneal clouding, retinal degeneration, blindness	Unknown; some patients with partial deficiency of ganglioside sialidase	*MCOLN1* gene at 19p13.2-13.3 encoding mucolipin 1; two founder variants accounting for 95% of mutant alleles in the Ashkenazi population	Increased in Ashkenazi Jews
Variable corneal clouding	β-Glucuronidase deficiency	*GUSB* gene at 7q21.2-q22; heterogeneous variants	Panethnic

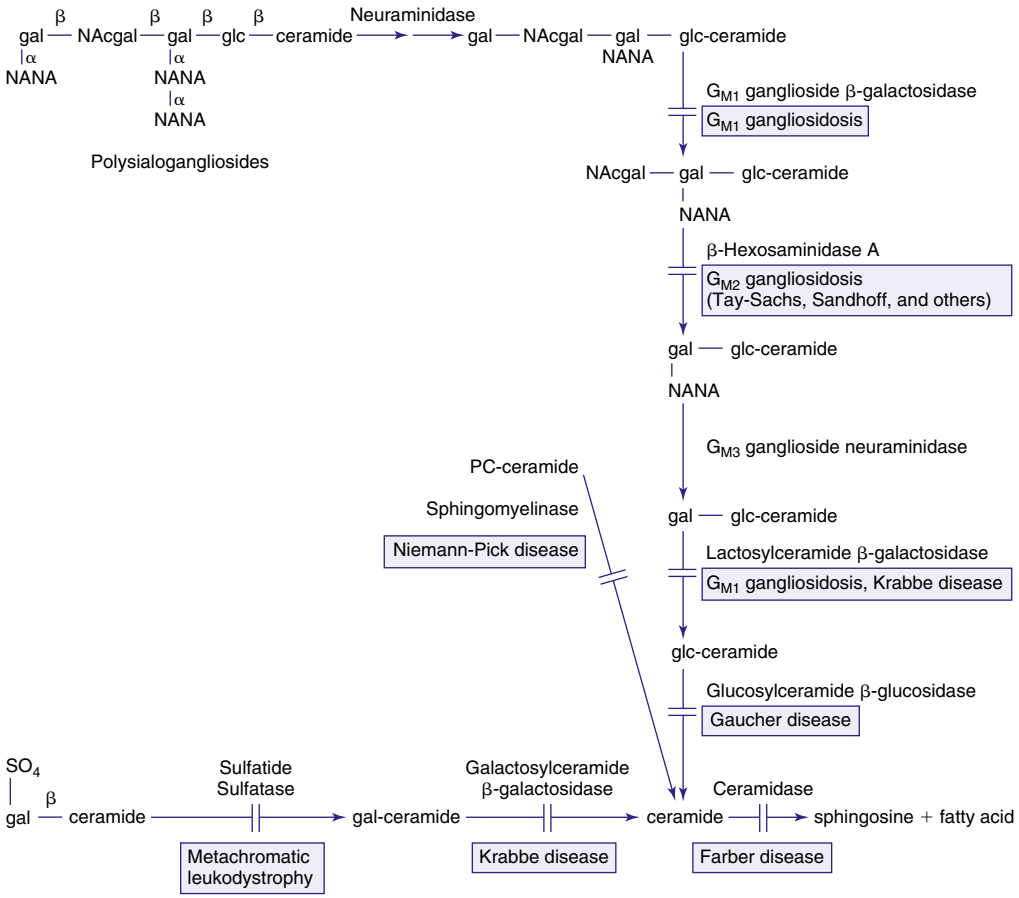

Fig. 106.15 Pathways in the metabolism of sphingolipids found in nervous tissues. The name of the enzyme catalyzing each reaction is given with the name of the substrate acted on. Inborn errors are depicted as bars crossing the reactions arrows, and the name of the associated defect or defects is given in the nearest box. The gangliosides are named according to the nomenclature of Svennerholm. Anomeric configurations are given only at the largest starting compound. Gal, galactose; glc, glucose; NAcgal, N-acetylgalactosamine; NANA, N-acetylneuraminic acid; PC, phosphorylcholine.

blood spots. There is no treatment available for Tay-Sachs disease or Sandhoff disease. The FDA has approved an investigational new drug application to initiate adeno-associated virus vector gene (beta hexosaminidase A and B) therapy for Tay-Sachs and Sandhoff disease.

GAUCHER DISEASE

This disease is a multisystemic lipidosis characterized by hematologic abnormalities, organomegaly, and skeletal involvement, the latter usually manifesting as bone pain and pathologic fractures (see Table 106.15 and 106.16). It is the most common lysosomal storage disease and the most prevalent genetic defect among Ashkenazi Jews. There are three clinical subtypes delineated by the absence or presence and progression of neurologic manifestations: type 1 or the adult, non-neuronopathic form; type 2, the infantile or acute neuronopathic form; and type 3, the juvenile or subacute neuronopathic form. All are autosomal recessive traits. Type 1, which accounts for 99% of cases, has a striking predilection for Ashkenazi Jews, with an incidence of about 1/1,000 and a carrier frequency of about 1/18.

Gaucher disease results from the deficient activity of the lysosomal hydrolase, acid β-glucosidase, which is encoded by the gene *GBA*. The enzymatic defect results in the accumulation of undegraded glycolipid substrates, particularly glucosylceramide, in cells of the reticuloendothelial system. This progressive deposition results in infiltration of the bone marrow, progressive hepatosplenomegaly, and skeletal complications. Four pathogenic variants—p.Asn370Ser, p.Leu444Pro, c.84insG, and IVS2+1G>A—account for about 95% of mutant alleles among Ashkenazi Jewish patients, permitting screening for this disorder in this population. Genotype-phenotype correlations have been noted, providing the molecular basis for the clinical heterogeneity seen in Gaucher disease type 1. Patients who are homozygous for the p.Asn370Ser pathogenic variant tend to have a later onset, with a more indolent course than patients with one copy of p.Asn370Ser and another common allele.

Clinical manifestations of type 1 Gaucher disease have a variable age at onset, from early childhood to late adulthood, with most symptomatic patients presenting by adolescence. At presentation, patients may have bruising from thrombocytopenia, chronic fatigue secondary to anemia, hepatomegaly with or without elevated LFT results, splenomegaly, and bone pain. Occasional patients have pulmonary involvement at the time of presentation. Patients presenting in the first decade frequently are not Jewish and have growth retardation and a more malignant course. Other patients may be discovered fortuitously during evaluation for other conditions or as part of routine examinations; these patients may have a milder or even a benign course. In symptomatic patients, splenomegaly is progressive and can become massive. Most patients develop radiologic evidence of skeletal involvement, including an Erlenmeyer flask deformity of the distal femur. Clinically apparent bony involvement, which occurs in most patients, can present as bone pain, a pseudo-osteomyelitis pattern, or pathologic fractures. Lytic lesions can develop in the long bones, including the femur, ribs, and pelvis; osteosclerosis may be evident at an early age. Bone crises with severe pain and swelling can occur. Bleeding secondary to thrombocytopenia may manifest as epistaxis or bruising and is frequently overlooked until other symptoms become apparent. With the exception of the severely growth-stunted child, who may experience developmental delay secondary to the effects of chronic disease, development and intelligence are normal.

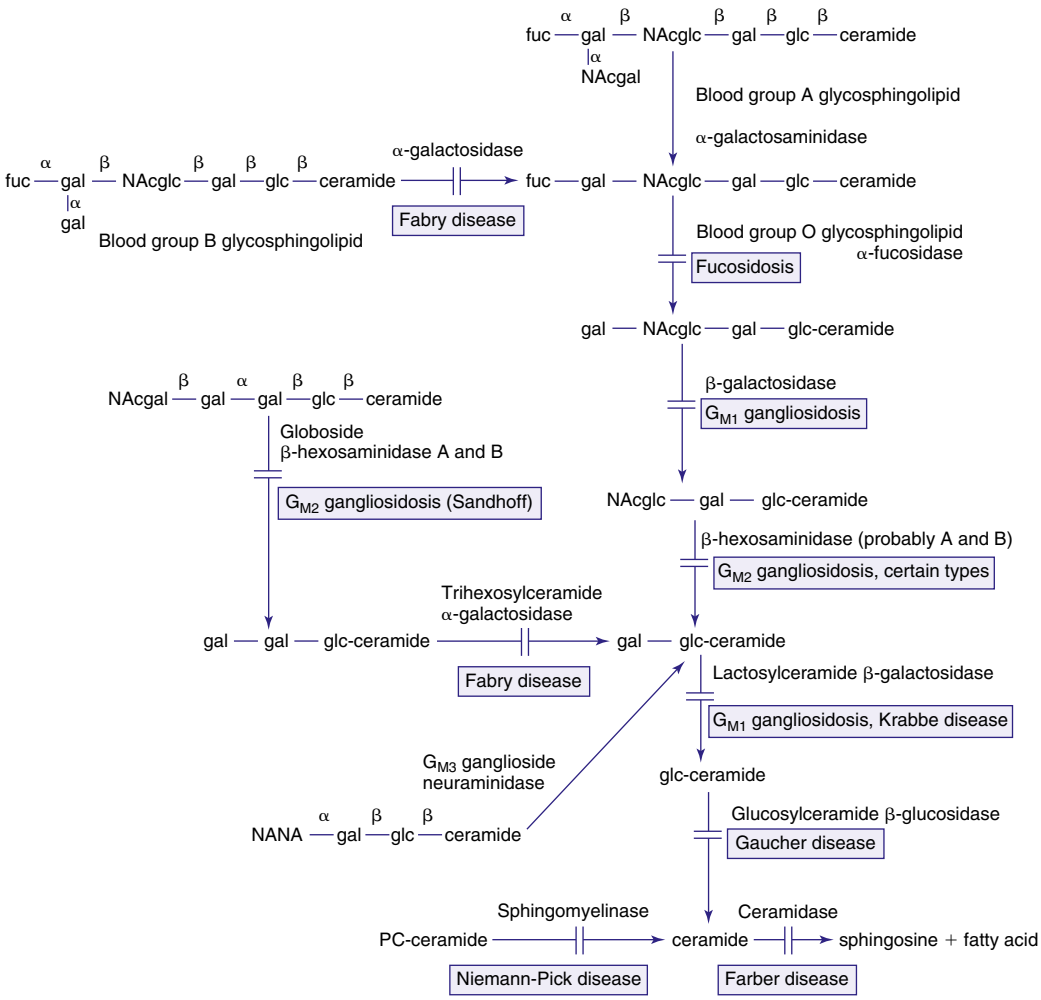

Fig. 106.16 Pathways in the degradation of sphingolipids found in visceral organs and red or white blood cells. See also the legend for Figure 106.15. Fuc, fucose; NAcglc, N-acetylglucosamine.

The pathologic hallmark of Gaucher disease is the Gaucher cell in the reticuloendothelial system, particularly in the bone marrow (Fig. 106.19). These cells, which are 20-100 μm in diameter, have a characteristic wrinkled paper appearance resulting from the presence of intracytoplasmic substrate inclusions. The cytoplasm of the Gaucher cell reacts strongly positive with the periodic acid–Schiff stain. The presence of this cell in bone marrow and tissue specimens is highly suggestive of Gaucher disease, although it also may be found in patients with granulocytic leukemia and myeloma.

Gaucher disease type 2 is much less common and does not have an ethnic predilection. It is characterized by a rapid neurodegenerative course with extensive visceral involvement and death within the first years of life. It presents in infancy with increased tone, strabismus, and organomegaly. Failure to thrive and stridor caused by laryngospasm are typical. After a several-year period of psychomotor regression, death occurs secondary to respiratory compromise. Gaucher disease type 3 presents as clinical manifestations that are intermediate to those seen in types 1 and 2, with a presentation in childhood and death by age 10-15 years. It has a predilection for the Swedish Norrbottnian population, among which the incidence is about 1/50,000. Neurologic involvement is present. Type 3 disease is further classified as types 3a and 3b based on the extent of neurologic involvement and whether there is progressive myotonia and dementia (type 3a) or isolated supranuclear gaze palsy (type 3b).

Gaucher disease should be considered in the differential diagnosis of patients with unexplained organomegaly, who bruise easily, have bone pain, or have a combination of these conditions. Bone marrow examination usually reveals the presence of Gaucher cells. All suspected diagnoses should be confirmed by genetic testing or by determination of the acid β-glucosidase activity in isolated leukocytes or cultured fibroblasts. In Ashkenazi Jewish individuals, the identification of carriers can be achieved best by molecular testing for the common pathogenic variants. Testing should be offered to all family members, keeping in mind that heterogeneity, even among members of the same kindred, can be so great that asymptomatic affected individuals may be diagnosed. Prenatal diagnosis is available by determination of the specific family pathogenic variants and/or enzyme activity of chorionic villi or cultured amniotic fluid cells.

Treatment of patients with Gaucher disease type 1 includes enzyme replacement therapy, with recombinant acid β-glucosidase (imiglucerase). Most extraskeletal symptoms (organomegaly, hematologic indices) are reversed by enzymes (60 IU/kg) administered by intravenous infusion every other week. Monthly maintenance enzyme replacement improves bone structure, decreases bone pain, and induces compensatory growth in affected children. A small number of patients have undergone bone marrow transplantation, which can be curative but results in significant morbidity and mortality from the procedure, making the selection of appropriate candidates limited. Although enzyme replacement does not alter the neurologic progression of patients with Gaucher disease types 2 and 3, it has been used in selected patients as a palliative measure, particularly in type 3 patients with severe visceral involvement. Alternative treatments, including the use of agents designed to decrease the synthesis of glucosylceramide by chemical inhibition of glucosylceramide synthase, are available for patients who cannot be treated by enzyme replacement.

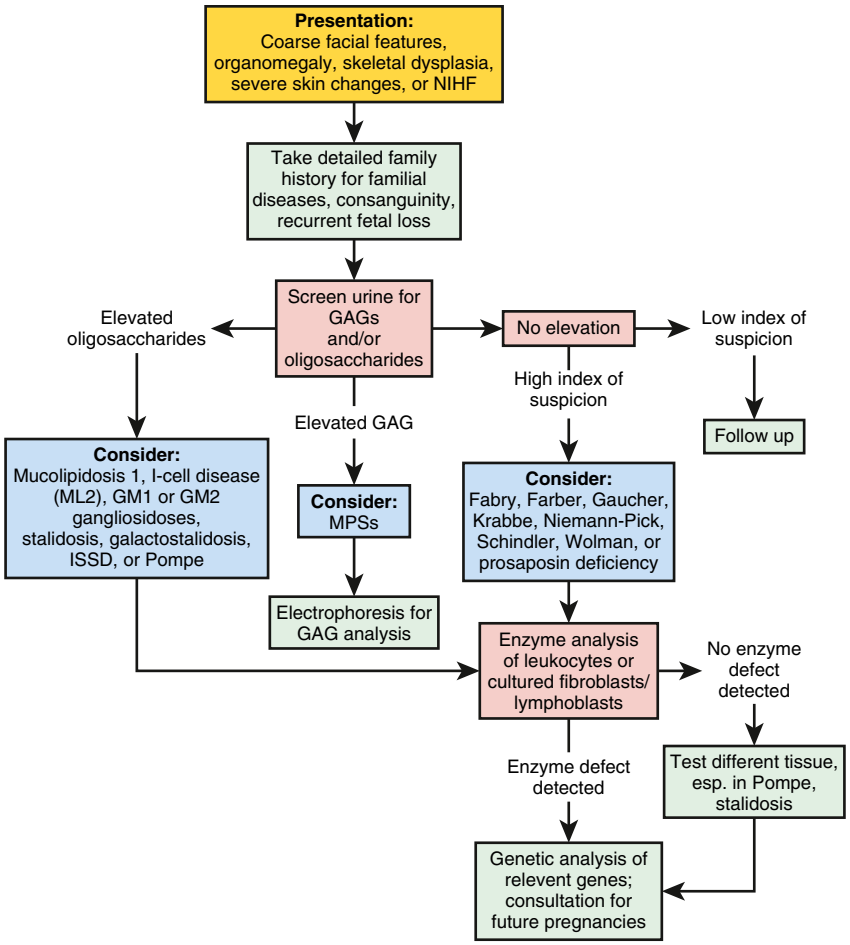

Fig. 106.17 Algorithm of the clinical evaluation recommended for an infant with a suspected lysosomal storage disease. GAGs, Glycosaminoglycans; NIHF, nonimmune hydrops fetalis. *(From Staretz-Chacham O, Lang TC, LaMarca ME, et al. Lysosomal storage disorders in the newborn. Pediatrics. 2009;123:1191–1207.)*

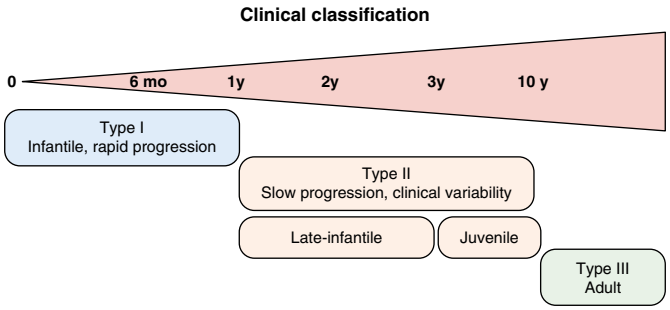

Fig. 106.18 Classification of GM1 gangliosidosis according to occurrence of the first symptom. *(From Arash-Kaps L, Komlosi K, Seegräber M, et al. The clinical and molecular spectrum of GM1 gangliosidosis. J Pediatr. 2019;215:152–157.)*

NIEMANN-PICK DISEASE

Type A NPD is a fatal disorder of infancy characterized by failure to thrive, hepatosplenomegaly, and a rapidly progressive neurodegenerative course that leads to death by 2-3 years of age. Type B disease is a non-neuronopathic form observed in children and adults. Type C disease is a neuronopathic form that results from defective cholesterol transport. All subtypes are inherited as autosomal recessive traits and display variable clinical features (see Tables 106.15 and 106.16).

NPD types A and B result from the deficient activity of acid sphingomyelinase, a lysosomal enzyme encoded by the gene *SMPD1*. The enzymatic defect results in the pathologic accumulation of sphingomyelin, a ceramide phospholipid, and other lipids in the monocyte-macrophage system as the primary pathologic site. The progressive deposition of sphingomyelin in the CNS results in the neurodegenerative course seen in type A and in non-neural tissue in the systemic disease manifestations of type B, including progressive lung disease in some patients. A variety of pathogenic variants in *SMPD1* that cause types A and B NPD have been identified.

The clinical manifestations and course of type A NPD is uniform and characterized by a normal appearance at birth. Hepatosplenomegaly, moderate lymphadenopathy, and psychomotor retardation are evident by 6 months of age, followed by neurodevelopmental regression and death by 3 years. With advancing age, the loss of motor function and the deterioration of intellectual capabilities are progressively debilitating with spasticity and rigidity and in later stages. Affected infants lose contact with their environment. In contrast to the stereotyped type A phenotype, the clinical presentation and course of patients with type B disease are more variable. Most are diagnosed in infancy or childhood when enlargement of the liver or spleen, or both, is detected during a routine physical examination. At diagnosis, type B NPD patients usually have evidence of mild pulmonary involvement, usually detected as a diffuse reticular or finely nodular infiltration on the chest radiograph. Pulmonary symptoms usually present in adulthood. In most patients, hepatosple-nomegaly is particularly prominent in childhood, but with increasing linear growth, the abdominal protuberance decreases and becomes less conspicuous. In mildly affected patients, the splenomegaly may not be noted until adulthood, and there may be minimal disease manifestations.

In some type B patients, decreased pulmonary diffusion caused by alveolar infiltration becomes evident in late childhood or early

Table 106.17 Symptoms and Biochemical Signs in Three Clinical Forms of GM_1 Gangliosidosis

SYMPTOMS AND SIGNS	INFANTILE FORM	LATE-INFANTILE FORM	JUVENILE FORM
CLINICAL SYMPTOMS			
Coarse facial features	+	–	–
Cherry red macula spot	–/+	–	–
Cardiomyopathy	–/+	–/+	–
Hepatosplenomegaly	–/+	–	–
Cognitive decline	+	+	+
Dystonia	–/+	–/+	–/+
Ataxia	–/+	–/+	–/+
Pyramidal signs	+	+	–
LABORATORY SIGNS			
Increased oligosaccharides	+	–/+	–/+
Increased ASAT	–/+	–/+	–
Increased chitotriosidase activity	>1,000	>100–1,000	na

na, Not applicable.
From Arash-Kaps L, Komlosi K, Seegräber M, et al. The clinical and molecular spectrum of GM1 gangliosidosis. *J Pediatr.* 2019;215:152–157, Table II.

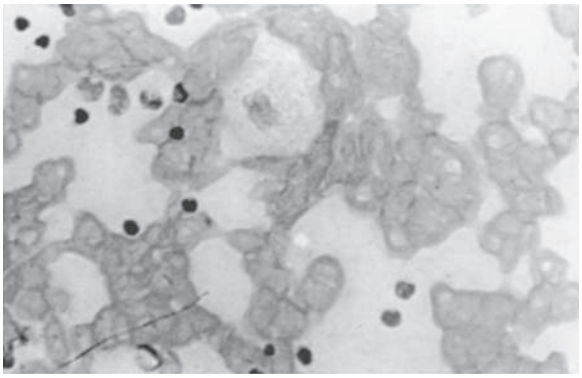

Fig. 106.19 Cells from the spleen of a patient with Gaucher disease. A characteristic spleen cell is shown engorged with glucocerebroside.

adulthood and progresses with age. Severely affected individuals may experience significant pulmonary compromise by 15-20 years of age. Such patients have low Po_2 values and dyspnea on exertion. Life-threatening bronchopneumonias may occur, and *cor pulmonale* has been described. Severely affected patients may have liver involvement leading to life-threatening cirrhosis, portal hypertension, and ascites. Clinically significant pancytopenia caused by secondary hypersplenism may require partial or complete splenectomy; this should be avoided if possible because splenectomy frequently causes progression of pulmonary disease, which can be life-threatening. In general, type B patients do not have neurologic involvement and have a normal IQ. Some patients with type B disease have cherry red maculae or haloes and subtle neurologic symptoms that can include peripheral neuropathy.

Type C NPD patients often present with prolonged neonatal jaundice, appear normal for 1-2 years, and then experience a slowly progressive and variable neurodegenerative course. Their hepato¬splenomegaly is less severe than that of patients with types A or B NPD, and they may survive into adulthood. The underlying biochemical defect in type C patients is an abnormality in cholesterol transport, leading to the accumulation of sphingomyelin and cholesterol in their lysosomes and a secondary partial reduction in acid sphingomyelinase activity (see Chapter 106.3).

In type B NPD patients, splenomegaly is usually the first manifestation detected. The splenic enlargement is noted in early childhood. In very mild disease, the enlargement may be subtle and detection may be delayed until adolescence or adulthood. The presence of the characteristic NPD cells in bone marrow aspirates supports the diagnosis of type B NPD. Patients with type C NPD, however, also have extensive infiltration of NPD cells in the bone marrow, and thus all suspected cases should be evaluated genetically or enzymatically to confirm the clinical diagnosis by measuring the acid sphingomyelinase activity level in peripheral leuko¬cytes, cultured fibroblasts, or lymphoblasts, or a combination of these cells. Patients with types A and B NPD have markedly decreased enzyme levels (1–10%), whereas patients with type C NPD have normal or mildly decreased acid sphingomyelinase activities. Without known genetic causes, the enzymatic identification of NPD carriers is problematic. In families in which the specific genetic lesion has been identified, however, family members can be accurately tested for heterozygote status by DNA analysis. Prenatal diagnosis of types A and B NPD can be made by genetic testing and reliably by the measurement of acid sphingomyelinase activity in cultured amniocytes or chorionic villi. Historically, the diagnosis of Niemann-Pick type C has relied on the demonstration of the cholesterol transport defect in cultured fibroblasts by filipin staining but is now readily accomplished by genetic testing of the *NPC1* and *NPC2* genes and the measurement of oxidative cholesterol metabolites. There is no conclusive treatment for types A and B NPD. Orthotopic liver transplantation in an infant with type A disease and amniotic cell transplantation in several type B NPD patients have been attempted with little or no success. Bone marrow transplantation in a small number of type B NPD patients has been shown to be successful in reducing the spleen and liver volumes, the sphingomyelin content of the liver, the number of Niemann-Pick cells in the marrow, and radiologically detected infiltration of the lungs. In one patient, liver biopsies taken up to 33 months after transplantation showed only a moderate reduction in stored sphingomyelin. Lung transplantation has not been performed in any severely compromised patient with type B disease, although two patients who underwent whole lung lavages with variable results have been reported. Enzyme replacement therapy (ERT) with recombinant human acid sphingomyelinase (olipudase alpha) is in clinical trials for the treatment of NPD type B. A 26-week phase 1b study in adult patients with NPD B established initial proof of concept in this patient group. In a phase 1/2 pediatric trial (ASCEND-Peds/NCT02292654) in children with chronic acid sphingomyelinase deficiency (ASMD), olipudase alfa was generally well-tolerated with improvements in disease pathology, and a phase 1/2 clinical trial in pediatric patients and a randomized phase 2/3 trial in adults (ASCEND) with ASMD documented improvement in lung function and spleen size.

Clinical trials of miglustat (Actelion, Basel, Switzerland) have been performed, and the drug has been approved in Europe for the treatment of type C disease. Treatment of type A disease with bone marrow transplantation has not been successful, presumably because of severe neurologic involvement.

FABRY DISEASE

Fabry disease is an X-linked inborn error of glycosphingolipid metabolism characterized by angiokeratomas (telangiectatic skin lesions); hypohidrosis; corneal and lenticular opacities; acroparesthesias; and vascular disease of the kidney, heart, and/or brain (see Table 106.15). The classic phenotype is caused by deficient activity of the enzyme α-galactosidase A and has an estimated prevalence of approximately 1/50,000 males. Later-onset affected males with residual α-galactosidase A activity may present with cardiac and/or renal disease, including hypertrophic cardio¬myopathy and renal failure, and is more prevalent than the classic phenotype. Heterozygous females for the classic phenotype can be either asymptomatic or as severely affected as the males—the variability is a result of random X-inactivation. The disease results from pathogenic variants in α-galactosidase A encoded by the X-linked *GLA* gene. The enzymatic defect leads to the systemic accumulation of neutral glycosphingolipids, primarily globotriaosylceramide, particularly in the plasma and lysosomes of vascular endothelial and smooth muscle cells. The progressive vascular glycosphingolipid deposition in classically affected males results in ischemia and infarction, leading to the major disease manifestations. The cDNA and genomic sequences encoding α-galactosidase A have identified more than 500 different pathogenic variants in the α-galactosidase A gene that are responsible for this lysosomal storage disease, including amino acid substitutions, gene rearrangements, and mRNA splicing defects.

Affected males with the classic phenotype have the skin lesions, acroparesthesias, hypohidrosis, and ocular changes, whereas males with the later-onset phenotypes lack these findings and present with cardiac and/or renal disease in adulthood (Table 106.18). The classic angiokeratomas usually occur in childhood and may lead to early diagnosis (Fig. 106.20). They increase in size and number with age and range from barely visible to several millimeters in diameter. The lesions are punctate, dark red to blue-black, and flat or slightly raised. They do not blanch with pressure, and the larger ones may show slight hyperkeratosis. Characteristically, the lesions are most dense between the umbilicus and knees, in the "bathing trunk area," but may occur anywhere, including the oral mucosa. The hips, thighs, buttocks, umbilicus, lower abdomen, scrotum, and glans penis are common sites, and there is a tendency toward symmetry. Variants without skin lesions have been described. Sweating is usually decreased or absent. Corneal opacities and characteristic lenticular lesions, observed under slit-lamp examination, are present in affected males and in about 90% of heterozygotes. Conjunctival and retinal vascular tortuosity is common and results from the systemic vascular involvement.

Pain is the most debilitating symptom in childhood and adolescence. Fabry crises, lasting from minutes to several days, consist of agonizing, burning pain in the hands, feet, and proximal extremities and are usually associated with exercise, fatigue, fever, or a combination of these factors. These painful acroparesthesias usually become less frequent in the third and fourth decades of life, although in some men, they may become more frequent and severe. Attacks of abdominal or flank pain may simulate appendicitis or renal colic.

The major morbid symptoms result from the progressive involvement of the vascular system. Early in the course of the disease, casts, red cells, and lipid inclusions with characteristic birefringent "Maltese crosses" appear in the urinary sediment. Proteinuria, isosthenuria, and gradual deterioration of renal function and development of azotemia occur in the second through fourth decades. Cardiovascular findings may include hypertension, left ventricular hypertrophy, anginal chest pain, myocardial ischemia or infarction, and heart failure. Mitral insufficiency is the most common valvular lesion. Abnormal electrocardiographic and echocardiographic findings are common. Cerebrovascular manifestations result from multifocal small vessel involvement. Other features may include chronic bronchitis and dyspnea, lymphedema of the legs without hypoproteinemia, episodic diarrhea, osteoporosis, retarded growth, and delayed puberty. Death most often results from uremia or vascular disease of the heart or brain. Before hemodialysis or renal transplantation, the mean age at death for affected men was 40 years. Patients with the later-onset phenotype with residual α-galactosidase A activity have cardiac and/or renal disease. The

Table 106.18 Summary of Reported Clinical Manifestations in Fabry Patients (Newborn to 4 Yr)

FABRY-RELATED SIGNS AND SYMPTOMS	EARLIEST REPORT OF SYMPTOM
Storage of globotriaosylceramide found in organs on biopsy	Prenatal
Corneal whorls/verticillata	Prenatal/newborn
Gastrointestinal problems, including nausea, vomiting, diarrhea, constipation, and abdominal pain	1.0 yr
Slow growth in boys (mean height/weight <50th percentile)	2.0 yr
Intermittent acroparesthesia/neuropathic pain triggered by stress, heat, fatigue, or exercise	2.0 yr
Hypohidrosis or anhidrosis	2.5 yr
Fabry crises of agonizing neuropathic pain typically begin in the hands and feet and may radiate proximally	2.5 yr
Heat, cold, and/or exercise intolerance	3.5 yr
Retinal vascular tortuosity	4.0 yr
Tinnitus/vertigo	4.0 yr
Low glomerular filtration rate	4.0 yr
T-wave inversion on electrocardiogram	4.0 yr
Trivial cardiac valve disease	4.0 yr
Angiokeratoma	4.4 yr

From Laney DA, Peck DS, Atherton AM, et al. Fabry disease in infancy and early childhood: A systematic literature review. *Genetics Med.* 2015;17(5):323–330, Table 2.

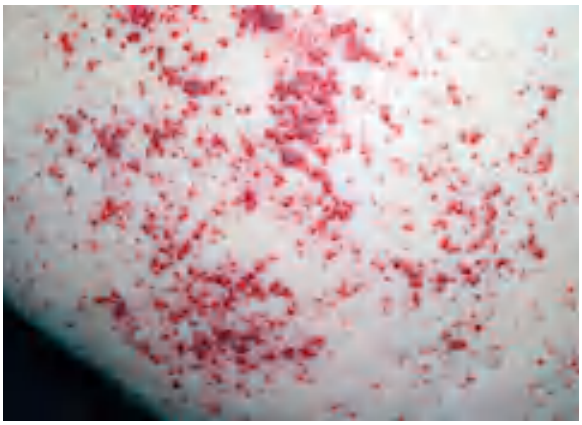

Fig. 106.20 Typical angiokeratomas. Angiokeratomas are quite large and easily recognizable, but if only a few lesions exist or they are restricted only to the genitals or umbilical regions, they can be easily missed. *(From Zarate VA, Hopkin RJ. Fabray's disease. Lancet. 2008;372:1427.)*

Table 106.19 Common Misdiagnoses for Fabry Disease

INFLAMMATORY
Systemic lupus erythematous
Rheumatic fever
Fibromyalgia
Dermatomyositis
Raynaud phenomenon
Raynaud syndrome
C1 esterase deficiency
TNF receptor–associated periodic syndrome (TRAPS)
Joint and recurrent fever syndromes (juvenile idiopathic arthritis, familial Mediterranean fever)
Erythromelalgia
NEUROLOGIC
Porphyria
Guillain-Barre syndrome
Hereditary neuropathies
Nutritional neuropathies
Uremic neuropathy
Diabetic neuropathy
Polyneuropathy
Meniere disease
Complex regional pain syndromes
Multiple sclerosis
Mitochondrial disorders
Migraine
MELAS
CADASIL
GASTROINTESTINAL/NUTRITION
Irritable bowel syndrome
Appendicitis
Metabolic bone disease (rickets, uremia, scurvy)
Crohn disease
Celiac disease
Peptic ulcer disease
OTHER
Growing pains
Chronic overlapping pain syndrome
Malingering
Coronary heart disease
Osler-Weber-Rendu disease
Cardiomyopathy
Gaucher disease

MELAS, Mitochondrial encephalopathy lactic acidosis, strokelike symptoms; CADASIL, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy.

cardiac manifestations include hypertrophy of the left ventricular wall and interventricular septum and electrocardiographic abnormalities consistent with cardiomyopathy. Others have had hypertrophic cardiomyopathy or myocardial infarction, or both.

The diagnosis in classically affected males is most readily made from the history of painful acroparesthesias, hypohidrosis, the presence of characteristic skin lesions, and the observation of the characteristic corneal opacities and lenticular lesions. The disorder is often misdiagnosed as rheumatic fever, erythromelalgia, or neurosis (Table 106.19). The skin lesions must be differentiated from the benign angiokeratomas of the scrotum (Fordyce disease) or from angiokeratoma circumscriptum. Angiokeratomas identical to those of Fabry disease have been reported in fucosidosis, aspartylglycosaminuria, late-onset GM_1 gangliosidosis, galactosialidosis, α-N-acetylgalactosaminidase deficiency, and sialidosis. Later-onset patients have been identified among patients on hemodialysis and among patients with hypertrophic cardiomyopathy or who have suffered cryptogenic strokes. Later-onset patients lack the early classic manifestations such as the angiokeratomas, acroparesthesias, hypohidrosis, and corneal opacities. The diagnosis of classic and later-onset patients is confirmed biochemically by the demonstration of markedly decreased α-galactosidase A activity in plasma, isolated leukocytes, or cultured fibroblasts or lymphoblasts.

Heterozygous females may have corneal opacities, isolated skin lesions, and intermediate activities of α-galactosidase A in plasma or cells. Rare female heterozygotes may have manifestations as severe as those in affected males. Asymptomatic at-risk females in families affected by Fabry disease, however, should be optimally diagnosed by the direct analysis of their family's specific pathogenic variant. Prenatal detection of affected males can be accomplished by the demonstration of deficient α-galactosidase A activity or the family's specific gene pathogenic variant in chorionic villi obtained in the first trimester or in cultured amniocytes obtained by amniocentesis in the second trimester of pregnancy. Fabry disease can be detected by newborn screening, and pilot studies have been conducted in Italy and Taiwan.

Treatment for Fabry disease may include the use of phenytoin and/or carbamazepine to decrease the frequency and severity of the chronic acroparesthesias and the periodic crises of excruciating pain. Renal transplantation and long-term hemodialysis are lifesaving procedures for patients with renal failure.

Recombinant α-galactosidase is a safe and effective ERT of choice for Fabry disease at a dose of 1 mg/kg every other week. It has been shown to clear microvascular endothelial deposits of globotriaosylceramide from the kidneys, heart, and skin in patients with Fabry disease with stabilization of renal disease, regression of hypertrophic cardiomyopathy, reduction of pain, and improvement in quality of life. Migalastat, a protein-folding chaperone therapy, is an oral treatment for Fabry that can both be used as a first-line therapy in ERT-naïve patients and as an alternative to ERT in patients with a migalastat-amenable pathogenic variant.

FUCOSIDOSIS

This is a rare autosomal recessive disorder caused by the deficient activity of α-fucosidase and the accumulation of fucose-containing glycosphingolipids, glycoproteins, and oligosaccharides in the lysosomes of the liver, brain, and other organs (see Table 106.15). α-Fucosidase is encoded by the *FUCA1* gene, and specific pathogenic variants are known. Although the disorder is panethnic, most affected patients are from Italy and the United States. There is wide variability in the clinical phenotype, with the most severely affected patients presenting in the first year of life with developmental delay and somatic features similar to those of the mucopolysaccharidoses. These features include frontal bossing, hepatosplenomegaly, facial features, and macroglossia. The CNS storage results in a relentless neurodegenerative course, with death in childhood. Patients with milder disease have angiokeratomas and longer survival. No specific therapy exists for the disorder, which can be diagnosed by the demonstration of pathogenic variants in *FUCA1* or deficient α-fucosidase activity in peripheral leukocytes or cultured fibroblasts. Carrier identification studies and prenatal diagnosis are possible by determination of the enzymatic activity or the specific family pathogenic variants.

SCHINDLER DISEASE

Schindler disease is an autosomal recessive neurodegenerative disorder that results from the deficient activity of α-N-acetylgalactosaminidase and the accumulation of sialylated and asialoglycopeptides and oligosaccharides (see Table 106.15). The enzyme is encoded by the gene *NAGA*. The disease is clinically heterogeneous, and two major phenotypes have been identified. Type I disease is an infantile-onset neuroaxonal dystrophy. Affected

infants have normal development for the first 9-15 months of life followed by a rapid neurodegenerative course that results in severe intellectual disability, cortical blindness, and frequent myoclonic seizures. Type II disease is characterized by a variable age at onset, mild retardation, and angiokeratomas. There is no specific therapy for either form of the disorder. The diagnosis is by demonstration of the enzymatic deficiency in leukocytes or cultured skin fibroblasts or specific gene pathogenic variants.

METACHROMATIC LEUKODYSTROPHY (MLD)

This is an autosomal recessive white matter disease caused by a deficiency of arylsulfatase A (ASA) encoded by the gene *ARSA,* which is required for the hydrolysis of sulfated glycosphingolipids. Another form of MLD is caused by a deficiency of a sphingolipid activator protein (SAP1) encoded by the gene *PSAP,* which is required for the formation of the substrate-enzyme complex. The deficiency of this enzymatic activity results in the white matter storage of sulfated glycosphingolipids, which leads to demyelination and a neurodegenerative course. Pathogenic variants fall into two groups that correlate with disease severity.

The clinical manifestations of the late infantile form of MLD, which is most common, usually present between 12 and 18 months of age as irritability, inability to walk, and hyperextension of the knee, causing genu recurvatum. The clinical progression of the disease relates to the pathologic involvement of both central and peripheral nervous systems, giving a mixture of upper and lower motor neuron and cognitive and psychiatric signs. Deep tendon reflexes are diminished or absent. Gradual muscle wasting, weakness, and hypotonia become evident and lead to a debilitated state. As the disease progresses, nystagmus, myoclonic seizures, optic atrophy, and quadriparesis appear, with death in the first decade of life (see Table 106.15). The juvenile form of the disorder has a more indolent course with an onset that may occur as late as 20 years of age. This form of the disease presents with gait disturbances, mental deterioration, urinary incontinence, and emotional difficulties. The adult form, which presents after the second decade, is similar to the juvenile form in its clinical manifestations, although emotional difficulties and psychosis are more prominent features. Dementia, seizures, diminished reflexes, and optic atrophy also occur in both the juvenile and adult forms. The pathologic hallmark of MLD is the deposition of metachromatic bodies, which stain strongly positive with periodic acid–Schiff and Alcian blue, in the white matter of the brain. Neuronal inclusions may be seen in the midbrain, pons, medulla, retina, and spinal cord; demyelination occurs in the peripheral nervous system. Bone marrow transplantation has resulted in normal enzymatic levels in peripheral blood but there is no clear evidence for clinical efficacy in terms of the neurologic course; supportive care remains the primary intervention. Lentiviral transduced ex vivo autologous stem and progenitor cells with human arylsulfatase cDNA treatment has resulted in sustained and clinically important benefits in children with early onset disease. Cognitive function has been preserved, as has motor development in preliminary trials.

The diagnosis of MLD should be suspected in patients with the clinical features of leukodystrophy. Decreased nerve con¬duction velocities, increased cerebrospinal fluid protein, metachromatic deposits in sampled segments of the sural nerve, and metachromatic granules in urinary sediment are all suggestive of MLD. Confirmation of the diagnosis is based on the demonstration of pathogenic variants in *ARSA* or reduced activity of ASA in leukocytes or cultured skin fibroblasts. Sphingolipid activator protein deficiency is diagnosed by genetic testing of *PSAP* or by measuring the concentration of SAP1 in cultured fibroblasts using a specific antibody to the protein. Carrier detection and prenatal diagnosis are available for all forms of the disorder.

MULTIPLE SULFATASE DEFICIENCY

This is an autosomal recessive disorder that results from the enzymatic deficiency of at least nine sulfatases including arylsulfatases A, B, and C and iduronate-2-sulfatase. The specific defect has been shown to be an enzyme in the C-α-formylglycine generating system encoded by the gene *SUMF1,* which introduces a common posttranslational modification in all of the affected sulfatases and explains the occurrence of these multiple enzyme defects. Because of the deficiency of these enzymes, sulfatides, mucopolysaccharides, steroid sulfates, and gangliosides accumulate in the cerebral cortex and visceral tissues, resulting in a clinical phenotype with features of leukodystrophy as well as those of the mucopolysaccharidoses. Severe ichthyosis may also occur. Carrier testing and prenatal diagnosis by measurement of the enzymatic activities can be performed. There is no specific treatment for multiple sulfatase deficiency other than supportive care.

KRABBE DISEASE

This condition, also called *globoid cell leukodystrophy,* is an autosomal recessive fatal disorder of infancy. It results from the deficiency of the enzymatic activity of galactocerebrosidase encoded by the *GALC* gene, leading to white matter accumulation of galactosylceramide, which is normally found almost exclusively in the myelin sheath. Both peripheral and central myelin are affected, resulting in spasticity and cognitive impairment coupled with deceptively normal or even absent deep tendon reflexes. Specific disease-causing pathogenic variants are known. The infantile form of Krabbe disease is rapidly progressive, and patients present in early infancy with irritability, seizures, and hypertonia (see Table 106.16). Optic atrophy is evident in the first years of life, and cognitive development is severely impaired. As the disease progresses, optic atrophy and severe developmental delay become apparent; affected children exhibit opisthotonos and typically die before 3 years of age. A second, late infantile form of Krabbe disease also exists, and patients present after the age of 2 years. Affected individuals, however, have a disease course similar to that of the early infantile form.

The diagnosis of Krabbe disease relies on the demonstration of the specific pathogenic variants or by enzymatic deficiency in white blood cells or cultured skin fibroblasts. Carrier identification and prenatal diagnosis are available. The development of methods to measure GALC activity on dried blood spots has led to the inclusion of Krabbe disease in the newborn screening programs of some states. Treatment of infants with Krabbe disease with umbilical cord blood cell transplantation has been reported in prenatally identified asymptomatic newborns and symptomatic infants. The long-term outcome of umbilical cord blood cell transplantation is being evaluated; transplanted infants develop neurologic manifestations at a slower rate but succumb to a neurologic demise.

FARBER DISEASE

This is a rare autosomal recessive disorder that results from the deficiency of the lysosomal enzyme acid ceramidase and the accumulation of ceramide in various tissues, especially the joints. Symptoms can begin as early as the first year of life with painful joint swelling and nodule formation (Fig. 106.21), which is sometimes diagnosed as rheumatoid arthritis. As the disease progresses, nodule or granulomatous formation on the vocal cords can lead to hoarseness and breathing difficulties; failure to thrive is common. In some patients, moderate CNS dysfunction is present (see Table 106.16). Patients may die of recurrent pneumonias in their teens; there is currently no specific therapy. The diagnosis of this disorder should be suspected in patients who have nodule formation over the joints but no other findings of rheumatoid arthritis. In such patients, ceramidase activity should be determined in cultured skin fibroblasts or peripheral leukocytes. Various disease-causing pathogenic variants have been identified in the gene encoding acid ceramidase *(ASAH1).* Carrier detection and prenatal diagnosis are

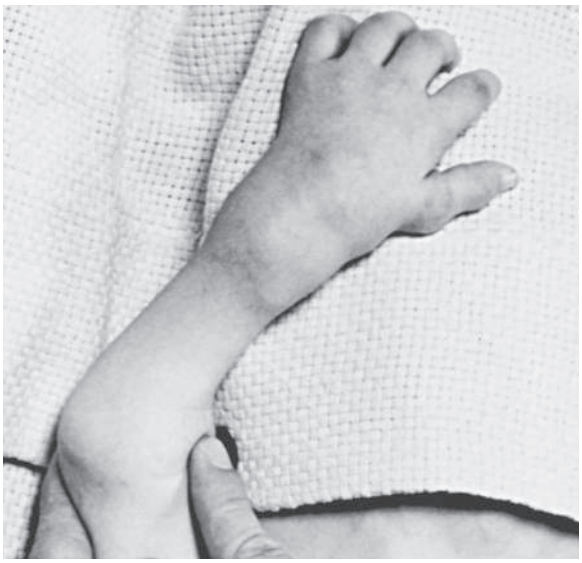

Fig. 106.21 Forearm of an 18-month old girl with Farber disease. Note the painful joint swelling and the nodule formation. The infant was suspected of having rheumatoid arthritis.

available. There is no specific treatment, but a recombinant human acid ceramidase is under development.

WOLMAN DISEASE AND CHOLESTEROL ESTER STORAGE DISEASE (CESD)

These are autosomal recessive lysosomal storage diseases that result from the deficiency of lysosomal acid lipase and the accumulation of cholesterol esters and triglycerides in histiocytic foam cells of most visceral organs. Wolman disease is the more severe clinical phenotype and is a fatal disorder of infancy. Clinical features become apparent in the first weeks of life and include failure to thrive, relentless vomiting, abdominal distention, steatorrhea, and hepatosplenomegaly (see Table 106.15). There usually is hyperlipidemia. Hepatic dysfunction and fibrosis may occur. Calcification of the adrenal glands is pathognomonic for the disorder. Death usually occurs within 6 months.

CESD is a less severe disorder that may not be diagnosed until adulthood. Hepatomegaly can be the only detectable abnormality, but affected individuals are at significant risk for premature atherosclerosis. Adrenal calcification is not a feature.

Lysosomal acid lipase is encoded by the *LIPA* gene. Diagnosis and carrier identification can be performed by genetic testing or by measuring acid lipase activity in peripheral leukocytes or cultured skin fibroblasts. Prenatal diagnosis can use pathogenic variants or enzyme levels in cultured chorionic villi or amniocytes. Pharmacologic agents to suppress cholesterol synthesis, in combination with cholestyramine and diet modification, have been used in patients with CESD (see Chapter 106.3). Sebelipase alfa (Kanuma) is a commercially available recombinant human lysosomal acid lipase that is approved in the European Union (EU), the United States, and Japan as a long-term ERT for patients diagnosed with LAL deficiency.

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106.5 Mucolipidoses

Margaret M. McGovern and Robert J. Desnick

I-cell disease (mucolipidosis II [ML-II]) and pseudo-Hurler polydystrophy (mucolipidosis III [ML-III]) are rare autosomal recessive disorders that share some clinical features with Hurler syndrome (see Chapter 109). These diseases result from the abnormal transport of newly synthesized lysosomal enzymes that are normally targeted to the lysosome by the presence of mannose-6-phosphate residues and recognized by specific lysosomal membrane receptors. These mannose-6-phosphate recognition markers are synthesized in a two-step reaction that occurs in the Golgi apparatus and is mediated by two enzymatic activities. The enzyme that catalyzes the first step, the UDP-N-acetylglucosamine:lysosomal enzyme *N*-acetylglucosamine-1-phosphotransferase, is defective in both ML-II and ML-III, which are allelic disorders resulting from pathogenic variants in the GlcNAc-phosphotransferase alpha/beta-subunits precursor gene *(GNPTAB)*. This enzyme deficiency results in the lack of mannose-6-phosphate tagging of lysosomal enzymes to result in disrupted targeting to the lysosome, and they are consequently secreted into the extracellular matrix. Because the lysosomal enzymes require the acidic medium of the lysosome to function, patients with this defect accumulate a variety of different substrates because of the intracellular deficiency of all lysosomal enzymes. The diagnosis of ML-II and ML-III can be made by genetic testing of the *GNPTAB* gene, determination of the serum lysosomal enzymatic activities, which are elevated, or by the demonstration of reduced enzymatic activity levels in cultured skin fibroblasts. Direct measurement of the phosphotransferase activity is possible as well. Prenatal diagnosis is available for both disorders using familial pathogenic variants or by measurement of lysosomal enzymatic activities in amniocytes or chorionic villus cells; carrier identification studies are available for both disorders using cultured skin fibroblasts. Neonatal screening by tandem mass spectroscopy may detect I-cell disease.

I-CELL DISEASE

This disorder shares many of the clinical manifestations of Hurler syndrome (see Chapter 109), although there is no mucopolysaccharidurria and the presentation is earlier (see Table 106.16). Some patients have clinical features evident at birth, including coarse facial features, craniofacial abnormalities, restricted joint movement, and hypotonia. Nonimmune hydrops may be present prenatally. The remainder of patients present in the first year with severe psychomotor retardation, coarse facial features, and skeletal manifestations that include kyphoscoliosis and a lumbar gibbus. Patients may also have congenital dislocation of the hips, inguinal hernias, and gingival hypertrophy. Progressive, severe intellectual disability leads to death in early childhood. No treatment is available.

PSEUDO-HURLER POLYDYSTROPHY

Pseudo-Hurler polydystrophy is a less severe disorder than I-cell disease, with later onset and survival to adulthood reported. Affected children may present around the age of 4 or 5 years of age with joint stiffness and short stature. Progressive destruction of the hip joints and moderate dysostosis multiplex are evident. Radiographic evidence of low iliac wings, flattening of the proximal femoral epiphyses with valgus deformity of the femoral head, and hypoplasia of the anterior third of the lumbar vertebrae are characteristic findings. Ophthalmic findings include corneal clouding, retinopathy, and astigmatism; visual complaints are uncommon (see Tables 106.15 and 106.16). Some patients have learning disabilities or mental retardation. Treatment, which should include orthopedic care, is symptomatic.

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Chapter 107

Defects in Metabolism of Carbohydrates

Ghada Hijazi and Priya S. Kishnani

Carbohydrate synthesis and degradation provide the energy required for most metabolic processes. The important carbohydrates include three monosaccharides—glucose, galactose, and fructose—and a polysaccharide, glycogen. Figure 107.1 shows the relevant biochemical pathways of these carbohydrates. **Glucose** is the principal substrate of energy metabolism, continuously available through dietary intake, glycogenolysis (breakdown of glycogen), and gluconeogenesis (glucose made de novo from amino acids, primarily alanine). Metabolism of glucose generates adenosine triphosphate (ATP) via glycolysis (conversion of glucose or glycogen to pyruvate), mitochondrial oxidative phosphorylation (conversion of pyruvate to carbon dioxide and water), or both. Dietary sources of glucose come from polysaccharides, primarily starch, and the disaccharides lactose, maltose, and sucrose. However, oral intake of glucose is intermittent and unreliable. Hepatic glycogenolysis provides the rapid release of glucose and is the most significant factor in maintaining euglycemia (normal levels of glucose in the blood). **Glycogen** is also the primary stored energy source in muscle, providing glucose for muscle activity during exercise. Gluconeogenesis contributes to maintaining euglycemia but is less immediate. Galactose and fructose are monosaccharides that provide fuel for cellular metabolism, though their role is less significant than that of glucose. **Galactose** is derived from lactose (galactose + glucose), which is found in milk and milk products. Galactose is an important energy source in infants. Galactose (exogenous or endogenously synthesized from glucose) is also an important component of certain glycolipids, glycoproteins, and glycosaminoglycans. The dietary sources of **fructose** are fruits, vegetables, and honey. It is also found in the disaccharide sucrose (fructose + glucose) and the sugar alcohol sorbitol.

Defects in glycogen metabolism typically cause an accumulation of glycogen in the tissues, thus the name **glycogen storage disease** (Table 107.1). Defects in gluconeogenesis or the glycolytic pathway, including galactose and fructose metabolism, do not result in an accumulation of glycogen (see Table 107.1). The defects in pyruvate metabolism in the pathway of the conversion of pyruvate to carbon dioxide and water via mitochondrial oxidative phosphorylation are more often associated with **lactic acidosis**.

107.1 Glycogen Storage Diseases

Ghada Hijazi and Priya S. Kishnani

The disorders of glycogen metabolism, the glycogen storage diseases (GSDs), result from deficiencies of enzymes or transport proteins in the pathways of glycogen metabolism (see Fig. 107.1). Glycogen found in these disorders is abnormal in quantity, quality, or both. GSDs are categorized by numerical type in accordance with the chronological order in which these enzymatic defects were identified. This numerical classification is still widely used, at least up to number VII. The GSDs can also be classified by organ involvement into liver and muscle glycogenoses (see Table 107.1).

There are more than 15 forms of GSDs. Glucose-6-phosphatase deficiency (type I), lysosomal acid α-glucosidase deficiency (type II), debrancher deficiency (type III), branching enzyme deficiency (type IV), liver phosphorylase deficiency (type VI), and liver phosphorylase kinase deficiency (type IX) are the most common of those that typically present in early childhood. Myophosphorylase deficiency (type V, McArdle disease) is the most common in adolescents and adults. The cumulative frequency of all forms of GSD is approximately 1 in 10,000-25,000 live births.

LIVER GLYCOGENOSES

The GSDs that principally affect the liver include glucose-6-phosphatase deficiency **(type I)**, debranching enzyme deficiency **(type III)**, branching enzyme deficiency **(type IV)**, liver phosphorylase deficiency **(type VI)**, phosphorylase kinase deficiency **(type IX)**, glycogen synthase deficiency **(type 0)**, and glucose transporter-2 (GLUT2) defect (Fanconi-Bickel syndrome). Because hepatic carbohydrate metabolism is responsible for plasma glucose homeostasis, this group of disorders typically causes fasting hypoglycemia and hepatomegaly. Some GSDs (types III, IV, VI, IX) can be associated with liver fibrosis and cirrhosis. Other organs can also be involved and may manifest as renal dysfunction in type I; myopathy (skeletal and/or cardiomyopathy) in types III, IV, and some rare forms of phosphorylase kinase deficiency; and neurologic involvement in types III (peripheral nerves) and IV (diffuse central and peripheral nervous system dysfunction).

Type I Glycogen Storage Disease (Glucose-6-Phosphatase or Translocase Deficiency, von Gierke Disease)

Type I GSD is caused by the absence or deficiency of **glucose-6-phosphatase activity** in the liver, kidney, and intestinal mucosa. It has two subtypes: **type Ia**, in which the defective enzyme is glucose-6-phosphatase, and **type Ib**, in which the defective enzyme is a **translocase** that transports glucose-6-phosphate across the microsomal membrane. Deficiency of the enzymes in both types Ia and Ib lead to inadequate hepatic conversion of glucose-6-phosphate to glucose through normal glycogenolysis and gluconeogenesis, resulting in fasting hypoglycemia.

Type I GSD is an autosomal recessive disorder. The gene for subtype Ia is *G6PC*, and the gene for subtype Ib is *SLC37A4*. Common pathogenic variants have been identified in different ancestral populations. Carrier detection and prenatal diagnosis are possible with DNA-based methodologies.

Clinical Manifestations

Patients with type I GSD may present in the neonatal period with hypoglycemia and lactic acidosis, but more often present at 3-4 months of age because of increased intervals between feeds and at time of weaning. Infants can present with hepatomegaly, hypoglycemic seizures, or both. Affected children are often described as having **"doll-like" faces** with full cheeks, relatively thin extremities, short stature, and a protuberant abdomen that is a consequence of massive hepatomegaly. The kidneys are also enlarged, whereas the spleen and heart are not involved.

Intermittent diarrhea may occur in GSD I. In patients with GSD Ib, diarrhea appears to be caused by the loss of mucosal barrier function via inflammation, which is likely related to glycogen-mediated impairment of neutrophil function. Easy bruising and epistaxis are common and are associated with a **prolonged bleeding time** as a result of impaired platelet aggregation and adhesion. A von Willebrand factor–like deficiency and/or dysfunction has also been found in GSD Ia patients.

The biochemical characteristics of type I GSD are hypoglycemia, lactic acidosis, hyperuricemia, and hyperlipidemia. Hypoglycemia and lactic acidosis can develop even after short fasts. Hyperuricemia is present in young children and rarely progresses to symptomatic gout before puberty. Despite marked hepatomegaly, the liver transaminase levels are usually normal or only slightly elevated. The plasma may be "milky" in appearance due to strikingly elevated triglyceride levels. Cholesterol and phospholipids are also elevated, albeit less prominently. The lipid abnormality resembles type IV hyperlipidemia and is characterized by increased levels of very low-density lipoprotein, low-density lipoprotein, and a unique apolipoprotein profile consisting of increased levels of apolipoproteins B, C, and E, with relatively normal or reduced levels of apolipoproteins A and D. The histologic appearance of the liver is characterized by a universal distention of hepatocytes by glycogen and

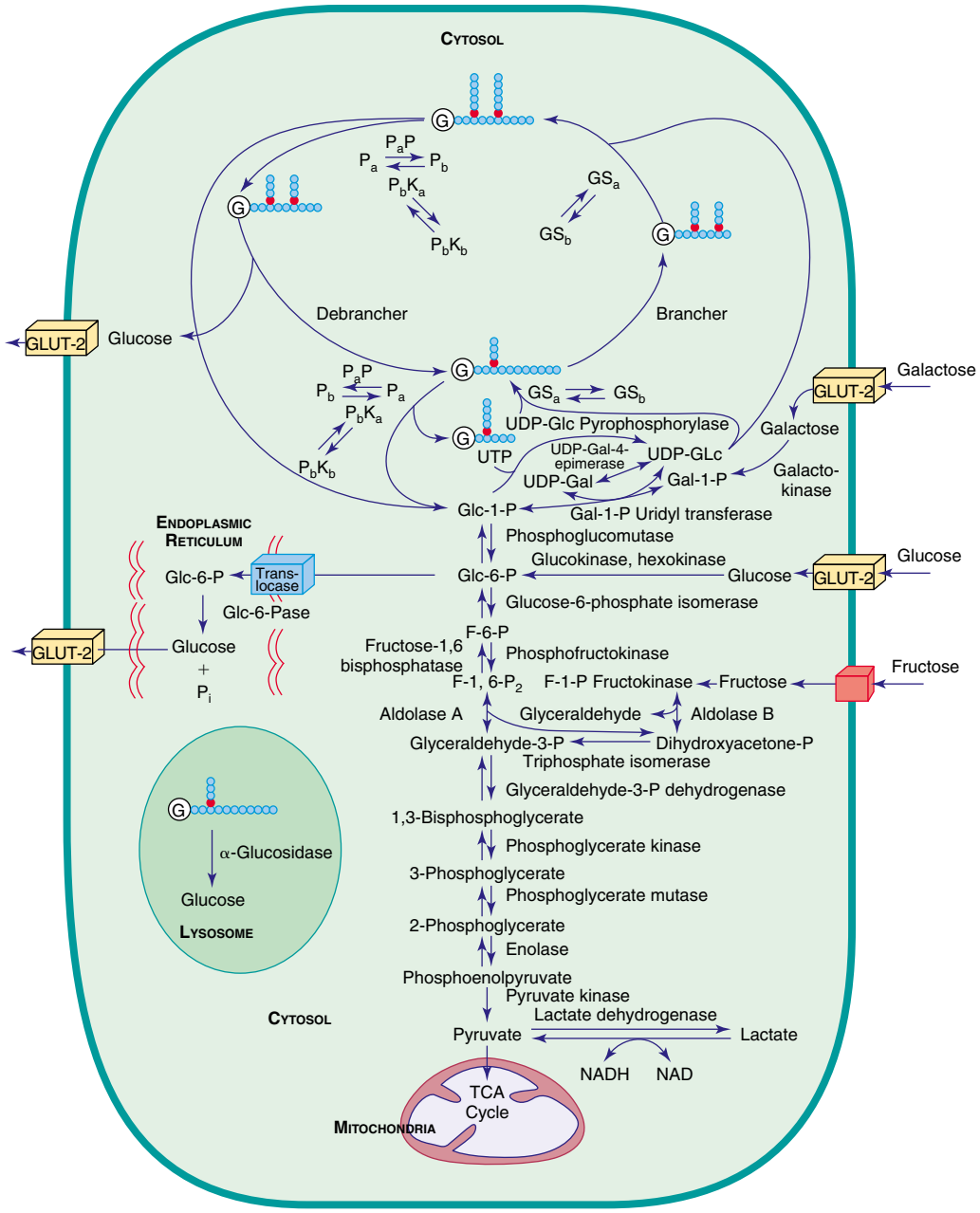

Fig. 107.1 Pathway related to glycogen storage diseases and galactose and fructose disorders. G, Glycogenin, the primer for glycogen synthesis; GLUT-2, glucose transporter 2; GSa, active glycogen synthase; GSb, inactive glycogen synthase; NAD/NADH, nicotinamide adenine dinucleotide; Pa, active phosphorylase; PaP, phosphorylase a phosphatase; Pb, inactive phosphorylase; PbKa, active phosphorylase b kinase; PbKb, inactive phosphorylase b kinase; UDP, uridine diphosphate. *(Adapted from Beaudet AR. Glycogen storage disease. In: Harrison TR, Isselbacher KJ, eds. Harrison's Principles of Internal Medicine, 13th ed. New York: McGraw-Hill; 1994.)*

fat. The lipid vacuoles are particularly large and prominent, and there is no associated liver fibrosis.

Although type I GSD affects mainly the liver, multiple organ systems are involved. **Delayed puberty** is often seen. Females can have ultrasound findings consistent with **polycystic ovaries**, even though other features of polycystic ovary syndrome (acne, hirsutism) are not seen. Nonetheless, fertility appears to be normal, as evidenced in several reports of successful pregnancy in women with GSD I. Increased bleeding during menstrual cycles, including life-threatening menorrhagia, has been reported and could be related to the impaired platelet aggregation. Symptoms of gout usually start around puberty from long-term hyperuricemia. There is an increased risk of **pancreatitis**, secondary to the lipid abnormalities. The dyslipidemia, together with elevated erythrocyte aggregation, could predispose these patients to atherosclerosis, but premature atherosclerosis has not yet been clearly documented except in rare cases. Impaired platelet aggregation and increased antioxidative defense to prevent lipid peroxidation may function as protective mechanisms to help reduce the risk of atherosclerosis. Frequent fractures and radiographic evidence of **osteopenia** are common, and bone mineral content is reduced, even in prepubertal patients.

By the second or third decade of life, some patients with type I GSD develop **hepatic adenomas** that can hemorrhage and become malignant in some cases. **Pulmonary hypertension** has been seen in some long-term survivors of the disease. Iron-refractory anemia and an increased prevalence of thyroid autoimmunity are also being recognized.

Renal disease is another late complication, and most patients with type I GSD over 20 years of age have proteinuria. Glomerular

Table 107.1 Features of the Disorders of Carbohydrate Metabolism

DISORDERS	BASIC DEFECTS	CLINICAL PRESENTATION	COMMENTS
LIVER GLYCOGENOSES			
Type/Common Name			
Ia/von Gierke	Glucose-6-phosphatase complex	Growth retardation, hepatomegaly, hypoglycemia; elevated blood lactate, cholesterol, triglyceride, and uric acid levels	Common, severe hypoglycemia. Adulthood: hepatic adenomas and risk for carcinoma, osteoporosis, pulmonary hypertension, and renal failure
Ib	Glucose-6-phosphate translocase	Same as type Ia, with additional findings of neutropenia, periodontal disease, inflammatory bowel disease, and increased risk of autoimmune hypothyroidism	10% of type I
IIIa/Cori or Forbes	Liver and muscle debrancher deficiency	Childhood: hepatomegaly, growth retardation, muscle weakness, hypoglycemia, hyperlipidemia, elevated transaminase levels, hepatic fibrosis, left ventricular hypertrophy. Later childhood to adulthood: muscle atrophy and weakness, liver cirrhosis and failure, risk for hepatocellular carcinoma, cardiac hypertrophy and fibrosis, life-threatening arrhythmia at any age.	Common, intermediate severity of hypoglycemia. Muscle weakness may progress to the point that ambulation assistance such as wheelchair use is required.
IIIb	Liver debrancher deficiency; normal muscle enzyme activity	Liver symptoms same as in type IIIa; no muscle or heart symptoms	15% of type III
IV/Andersen/adult polyglucosan body disease	Branching enzyme	Infancy/childhood failure to thrive, hypotonia, hepatomegaly, splenomegaly, cirrhosis progressing to liver failure (death usually before fifth year), elevated transaminase levels; a subset does not have overt progression to liver failure. Risk for extrahepatic manifestations, such as myopathy and cardiomyopathy. Perinatal: decreased fetal movements, polyhydramnios, fetal hydrops; arthrogryposis, hypotonia, muscle atrophy at birth, death in the perinatal period. Congenital: neonatal hypotonia, respiratory failure, dilated cardiomyopathy, early infantile death. Childhood: neuromuscular manifestations, cardiomyopathy. Adult form, adult polyglucosan body disease (APBD): progressive muscle weakness, neuropathic bladder, gait spasticity, central and peripheral nervous system involvement.	A continuum of disease severity, related to residual enzyme activity; multisystem involvement
VI/Hers	Liver phosphorylase	Hepatomegaly, hypoglycemia, hyperlipidemia, elevated liver enzyme levels, and ketosis	Often underdiagnosed; severe presentation also known
IX/phosphorylase kinase (PhK) deficiency			Common, X-linked GSD IX α2, typically less severe than autosomal form GSD IX γ2; clinical variability within and between subtypes; severe cases being recognized across different subtypes
IX α2 (*PHKA2* variant)	Liver PhK	Hypoglycemia, hyperketosis, hepatomegaly, chronic liver disease, liver fibrosis, hyperlipidemia, elevated liver enzymes, growth retardation	X-linked
IX β (*PHKB* variant)	Liver and muscle PhK	Hepatomegaly, growth retardation, mild or absent muscle disease.	Autosomal recessive
IX γ2 (*PHKG2* variant)	Liver PhK	More severe than IX α2; marked hepatomegaly, recurrent hypoglycemia, hyperlipidemia, markedly elevated liver enzymes, liver fibrosis/cirrhosis	Autosomal recessive
0a/Liver glycogen synthase deficiency	Liver glycogen synthase	Early morning drowsiness and fatigue, fasting hypoglycemia and ketosis, no hepatomegaly	Decreased liver glycogen stores
XI/Fanconi-Bickel syndrome	Glucose transporter 2 (GLUT-2)	Failure to thrive, rickets, hepatomegaly, renomegaly, proximal renal tubular dysfunction, impaired glucose and galactose use	GLUT-2 expressed in liver, kidney, pancreas, and intestine
MUSCLE GLYCOGENOSES			
Type/Common Name			
II/infantile Pompe	Acid α-glucosidase	Cardiomegaly, hypotonia, hepatomegaly; onset at birth to age 6 mo	Common, cardiorespiratory failure leading to death by age 1-2 yr; minimal to no residual enzyme activity
II/late-onset Pompe (juvenile and adult)	Acid α-glucosidase	Myopathy (proximal limb girdle), variable cardiomyopathy, respiratory insufficiency; onset from infancy and early childhood to adulthood	Residual enzyme activity
Danon disease	Lysosome-associated membrane protein 2 (LAMP2)	Hypertrophic cardiomyopathy, heart failure, retinopathy, mild cognitive dysfunction	Rare, X-linked

Table 107.1 Features of the Disorders of Carbohydrate Metabolism—cont'd

DISORDERS	BASIC DEFECTS	CLINICAL PRESENTATION	COMMENTS
PRKAG2 syndrome	Adenosine monophosphate (AMP)–activated protein kinase γ	Hypertrophic cardiomyopathy, arrhythmias, myopathy, myalgia, seizures; congenital fetal form is rapidly fatal	Autosomal dominant
0b/muscle glycogen synthase deficiency	Muscle glycogen synthase	Muscle weakness, hypertrophic cardiomyopathy, abnormal heart rate, sudden cardiac arrest	Rare, few cases reported
XV/Polyglucosan body myopathy 2	Glycogenin-1	Juvenile- or adult-onset proximal muscle weakness, nervous system involvement uncommon	Rare, autosomal recessive
V/McArdle	Myophosphorylase	Exercise intolerance, muscle cramps, myoglobinuria, "second-wind" phenomenon, muscle weakness	Common, male predominance
VII/Tarui	Phosphofructokinase	Exercise intolerance, muscle cramps, compensatory hemolytic anemia, myoglobinuria, "out-of-wind" phenomenon	Prevalent in Japanese and Ashkenazi Jewish populations
IX α1 (*PHKA1* variant)	Muscle PhK	Exercise intolerance, cramps, myalgia, increased CK, myoglobinuria in some; no hepatomegaly	X-linked
Phosphoglycerate kinase deficiency	Phosphoglycerate kinase	As with type V	Rare, X-linked
X/Phosphoglycerate mutase deficiency	M subunit of phosphoglycerate mutase	As with type V	Rare, majority of patients are Black
XI/Lactate dehydrogenase deficiency	M subunit of lactate dehydrogenase	As with type V	Rare
GALACTOSE DISORDERS			
Galactosemia with transferase deficiency	Galactose-1-phosphate uridyltransferase	Vomiting, feeding difficulties, liver failure/dysfunction/hepatomegaly, cataracts, failure to thrive, *E. coli* sepsis, generalized aminoaciduria	Black patients tend to have milder symptoms
Galactokinase deficiency	Galactokinase	Cataracts	Benign
Generalized uridine diphosphate galactose-4-epimerase deficiency	Uridine diphosphate galactose-4-epimerase	Similar to transferase deficiency with additional findings of hypotonia and nerve deafness	Benign and intermediate variants also exist
Galactose mutarotase deficiency	Galactose mutarotase	Cataract	Benign
FRUCTOSE DISORDERS			
Essential fructosuria	Fructokinase	Urine-reducing substance	Benign
Hereditary fructose intolerance	Fructose-1-phosphate aldolase (aldolase-B)	Acute; vomiting, hypoglycemia, sweating, lethargy, acute liver failure/liver dysfunction Chronic; failure to thrive, hepatic failure	Prognosis good with fructose restriction
DISORDERS OF GLUCONEOGENESIS			
Fructose-1,6-bisphosphatase deficiency	Fructose-1,6-diphosphatase	Episodic hypoglycemia, lactic acidosis, liver failure/dysfunction	Good prognosis, avoid fasting
Phosphoenolpyruvate carboxykinase deficiency	Phosphoenolpyruvate carboxykinase	Hypoglycemia, hepatomegaly, hypotonia, failure to thrive	Rare
DISORDERS OF PYRUVATE METABOLISM			
Pyruvate dehydrogenase complex defect	Pyruvate dehydrogenase	Severe fatal neonatal to mild late onset, lactic acidosis, psychomotor retardation, failure to thrive, features overlapping fetal alcohol syndrome, MRI findings suggestive of Leigh syndrome, ataxia	Most commonly caused by $E_{1\alpha}$ subunit defect, X-linked
Pyruvate carboxylase deficiency	Pyruvate carboxylase	Type B: neonatal severe lactic acidosis, hyperammonemia, hypercitrullinemia, hyperlysinemia, and hypoglycemia. Type A: late-onset mild to moderate lactic acidosis and developmental delay. Type C: recurrent episodes of lactic acidosis, ketoacidosis, and mild neurologic deficits.	Rare, autosomal recessive
Respiratory chain defects (oxidative phosphorylation disease)	Complexes I-V, many mitochondrial and nuclear DNA variants	Heterogeneous with multisystem involvement (see Table 107.3 and Table 107.5)	Mitochondrial, autosomal recessive, dominant, and X-linked inheritance
DISORDERS IN PENTOSE METABOLISM			
Pentosuria	l-Xylulose reductase	Urine-reducing substance	Benign
Transaldolase deficiency	Transaldolase	Intrauterine growth restriction, oligohydramnios, dysmorphism, cardiovascular anomalies, anemia, thrombocytopenia, hepatosplenomegaly, endocrine abnormalities, cutis laxa	Autosomal recessive, milder form exists
Ribose-5-phosphate isomerase deficiency	Ribose-5-phosphate isomerase	Progressive leukoencephalopathy and peripheral neuropathy	Four cases reported

hyperfiltration, increased renal plasma flow, and microalbuminuria are often found in the early stages of renal dysfunction and can occur before the onset of proteinuria. In younger patients, hyperfiltration and hyperperfusion may be the only signs of renal abnormalities. Many also have hypertension, renal stones, nephrocalcinosis, proteinuria, renal tubular acidosis (proximal and distal renal acidification defects), and altered creatinine clearance. With the advancement of renal disease, focal segmental glomerulosclerosis and interstitial fibrosis become evident. In some patients, renal function has deteriorated and progressed to failure, requiring dialysis and transplantation. Other renal abnormalities include amyloidosis, a Fanconi-like syndrome, hypocitraturia, hypercalciuria, and a distal renal tubular acidification defect. Patients with *GSD Ib* can have additional features of recurrent bacterial infections from **neutropenia** and impaired neutrophil function. Oral involvement, including recurrent mucosal ulceration, gingivitis, and rapidly progressive periodontal disease, may occur in type Ib. Intestinal mucosa ulceration culminating in GSD enterocolitis is also common. Type 1b is also associated with a chronic inflammatory bowel disease (IBD)–like picture involving the colon that may be associated with neutropenia and/or neutrophil dysfunction and can resemble ulcerative colitis or Crohn disease.

Diagnosis

The clinical presentation and laboratory findings of hypoglycemia, lactic acidosis, hyperuricemia, and hyperlipidemia lead to a suspected diagnosis of type I GSD. Neutropenia is noted in *GSD Ib* patients, although neutrophil counts may be normal initially. Neutropenia has also been noted in some patients with GSD Ia, especially those with the p.G188A variant. Administration of glucagon or epinephrine leads to a negligible increase, if any, in blood glucose levels, but the lactate level rises significantly. Before the availability of genetic testing, a definitive diagnosis required enzyme testing via liver biopsy. Gene-based variant analysis using single-gene sequencing or multigene panel testing provides a noninvasive way to diagnose most patients with GSD types Ia and Ib and has become the primary recommended method of diagnosis.

Treatment

Treatment focuses on maintaining normal blood glucose levels and is achieved by continuous nasogastric (NG) infusion of glucose or administration of uncooked cornstarch. In infancy, overnight NG drip feeding may be needed to maintain normoglycemia. NG feedings can consist of a sucrose- and lactose-free enteral formula or only glucose or a glucose polymer to provide sufficient glucose to maintain euglycemia. During the day, frequent feedings with high-complex carbohydrate content are typically sufficient.

Uncooked cornstarch acts as a slow-release form of glucose and can be introduced at a dose of 1.6 g/kg every 4 hours for children less than 2 years of age. The response of young children is variable. For older children, the cornstarch regimen can be changed to every 4-6 hours at a dose of 1.6-2.5 g/kg body weight and can be given orally mixed with water or a sucrose-, fructose-, and lactose-free beverage. Cornstarch dosing may also be calculated based on hepatic glucose production rate and can be administered more frequently in smaller amounts to improve metabolic control. Other starch products, such as extended-release waxy maize starch with a high amylopectin content, are shown to be beneficial in extending time between overnight feedings in some individuals with hepatic GSD. Because fructose and galactose cannot be converted directly to glucose in GSD type I, these sugars should be restricted in the diet. Sucrose (table sugar, cane sugar, other ingredients), fructose (fruit, juice, high-fructose corn syrup), lactose (dairy foods), and sorbitol should be avoided or limited. As a result of these dietary restrictions, vitamins and minerals such as calcium and vitamin D may be deficient, and supplementation is required to prevent nutritional deficiencies.

Maintaining normoglycemia and avoiding hypoglycemia necessitate frequent blood glucose measurements in patients with hepatic GSDs. This can be accomplished by self-monitoring blood glucose (SMBG) using a finger prick before and after meals, before physical activity, and overnight. Continuous glucose monitoring systems (CGMSs) are a newer technique that allow for 24-hour glucose monitoring. CGMSs have been shown to be safe and effective tools for monitoring glucose levels in patients with hepatic GSDs (GSD I, GSD III, GSD VI, GSD IX) at all hours of the day. Furthermore, when accompanied with the necessary dietary changes, CGMSs aid in metabolic control, improving disease parameters, avoidance of hospitalizations, and blood glucose stability in these patients.

Dietary therapy improves hyperuricemia, hyperlipidemia, and renal function, as well as slowing the development of renal failure. Blood uric acid and lipid levels may, however, be elevated in some individuals, despite good dietary compliance, especially after puberty. The control of hyperuricemia can be further augmented by the use of allopurinol, a xanthine oxidase inhibitor. Hyperlipidemia can be reduced with lipid-lowering drugs, such as β-hydroxy-β-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors and fibrate (see Chapter 106). **Microalbuminuria**, an early indicator of renal dysfunction in type I disease, is treated with angiotensin-converting enzyme (ACE) inhibitors. Citrate supplements can be beneficial for patients with hypocitraturia by preventing or ameliorating nephrocalcinosis and development of urinary calculi. Products containing citric acid reduce the effectiveness of cornstarch and should not be taken concurrently. Thiazide diuretics increase renal reabsorption of filtered calcium and decrease urinary calcium excretion, thereby preventing hypercalciuria and nephrocalcinosis. Growth hormone (GH) should be used with extreme caution and limited to only those with a documented GH deficiency. Even in those patients, there should be close monitoring of metabolic parameters and for the presence of adenomas. Kidney transplantation may be performed to treat end-stage renal disease (ESRD). As needed, combined liver and kidney transplantation is sometimes recommended.

In patients with **type Ib GSD**, granulocyte colony-stimulating factor is successful in correcting neutropenia, decreasing the number and severity of bacterial infections, and improving chronic IBD; the minimum effective dose should be used, given noted side effects with this agent, including splenomegaly, hypersplenism, and bone pain, with this agent. Vitamin E administration has been shown to improve neutrophil count and function and to reduce the frequency and severity of infections in GSD1b patients. The use of sodium glucose cotransporter-2 (SGLT2) inhibitors (empagliflozin) to improve neutrophil function and reduce neutropenia and IBD symptoms in GSDIb is showing promising results. Bone marrow transplantation has been reported to correct neutropenia in type Ib GSD.

Orthotopic liver transplantation is a potential cure for type I GSD, especially for patients with liver malignancy, multiple liver adenomas, metabolic derangements refractory to medical management, and liver failure; however, because of the paucity of available organ donors, this can be challenging. Large adenomas (>2 cm) that are rapidly increasing in size and/or number may necessitate partial hepatic resection. Smaller adenomas (<2 cm) may be treated with percutaneous ethanol injection or transcatheter arterial embolization. Recurrence of liver adenomas is a challenge and may potentiate malignant transformation in these patients, ultimately requiring a liver transplant. Before any surgical procedure, the bleeding status must be evaluated and good metabolic control established. Prolonged bleeding times can be normalized with intensive intravenous (IV) glucose infusion for 24-48 hours before surgery. Treatment with DDAVP (1-deamino-8-d-arginine vasopressin) can reduce bleeding complications, but it should be used with caution because of the risk of fluid overload and hyponatremia when administered as an IV infusion. Lactated Ringer solution should be avoided because it contains lactate and no glucose. Glucose levels should be maintained in the normal range throughout surgery with the use of 10% dextrose. Overall, metabolic control is assessed by growth, improvement, and correction of the metabolic abnormalities such as elevated lactate, glucose, triglyceride, cholesterol, and uric acid levels.

GSD I is a promising target for gene therapy because it is caused by pathogenic variants in a single gene. An active phase 3 clinical trial (NCT05139316) is being conducted to determine the efficacy and safety of adeno-associated virus serotype 8 (AAV8)–mediated G6P gene replacement in patients ages 8 years and older with GSD type Ia.

Prognosis

Inadequate metabolic control during childhood can lead to long-term complications in adults. Clinical outcomes have improved dramatically with early diagnosis and effective treatment. However, serious complications such as renal disease and formation of hepatic adenomas with potential risk for malignant transformation persist. The ability to identify transformation of liver adenomas to hepatocellular carcinoma remains a challenge as α-fetoprotein (AFP) and carcinoembryonic antigen (CEA) levels often remain normal in the setting of hepatocellular carcinoma.

Type III Glycogen Storage Disease (Cori Disease, Forbes Disease, Debrancher Deficiency, Limit Dextrinosis)

Type III GSD is caused by deficient activity of the **glycogen debranching enzyme**. This enzyme, together with phosphorylase and phosphorylase kinase, are responsible for complete degradation of glycogen. When debranching enzyme is defective, glycogen breakdown is incomplete, resulting in the accumulation of an abnormal glycogen with short outer-branch chains that resemble *limit dextrin*. GSD **type IIIa** is the most common type and usually involves both liver and muscle; GSD **type IIIb**, seen in approximately 15% of patients, appears to involve only the liver.

Type III GSD is an autosomal recessive disease that has been reported in many different ethnic groups. The frequency is relatively high in the Sephardic Jewish population from North Africa, inhabitants of the Faroe Islands, and the Inuit population. The gene for debranching enzyme *(AGL)* has been noted, with more than 260 different pathogenic variants. At least two pathogenic variants in exon 2, c.18_19del (p.Gln6fs) and c.16C>T (p.Gln6Ter), are specifically associated with GSD type IIIb. Carrier detection and prenatal diagnosis are possible using DNA-based testing.

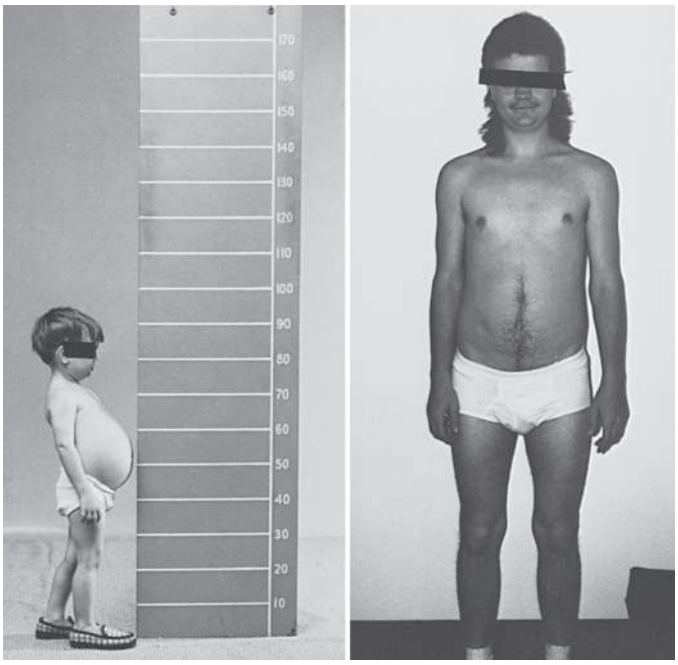

Fig. 107.2 Growth and development in a patient with type IIIb glycogen storage disease. The patient has debrancher deficiency in the liver but normal activity in muscle. As a child, he had hepatomegaly, hypoglycemia, and growth retardation. After puberty, he no longer had hepatomegaly or hypoglycemia, and his final adult height is normal. He had no muscle weakness or atrophy; this is in contrast to patients with type IIIa, in whom a progressive myopathy is seen in adulthood.

Clinical Manifestations

In infancy and childhood, type III GSD may be indistinguishable from type I because of overlapping features such as hepatomegaly, hypoglycemia, hyperlipidemia, and growth stunting (Fig. 107.2). Hepatomegaly in most patients with type III GSD decreases with age; however, liver fibrosis and/or cirrhosis progressing to liver failure are noted in many in late adulthood. Some patients develop hepatocellular carcinoma. Hepatic adenomas with transformation to carcinomas occur less frequently in individuals with GSD III than in those with GSD I. Alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) levels are not good predictors of malignant transformation.

In patients with GSD type IIIa, the **muscle weakness** is slowly progressive and associated with muscle wasting. The weakness can present in early childhood but can become severe after the third or fourth decade of life. Myopathy does not follow a particular pattern of involvement, and both proximal and distal muscles are involved. Electromyography (EMG) reveals a widespread myopathy, and nerve conduction studies may be abnormal.

Although overt cardiac dysfunction is rare, ventricular hypertrophy is a frequent finding. **Cardiac pathology** has shown diffuse involvement of various cardiac structures, including vacuolation of myocytes, fibrosis, glycogen accumulation in the conduction system, and hyperplasia of smooth muscles. Life-threatening arrhythmia and the need for heart transplant have been reported in some patients with GSD IIIa. Hepatic symptoms in some patients may be mild and the diagnosis is not made until adulthood, when patients show symptoms and signs of neuromuscular disease.

The initial presentation can be confused with Charcot-Marie-Tooth disease (see Chapter 653.1). Polycystic ovaries are noted; some patients can develop hirsutism, irregular menstrual cycles, and other features of polycystic ovarian syndrome. Fertility does not appear to be affected; successful pregnancies have been reported. Low bone mineral density and myopathy in patients with GSD IIIa put them at an increased risk of potential fractures.

Hypoglycemia and hyperlipidemia are common. In contrast to type I GSD, elevation of liver transaminase levels and fasting ketosis are prominent, but blood lactate and uric acid concentrations are usually normal. Glucagon administration 2 hours after a carbohydrate meal provokes a normal increase in blood glucose. However, after an overnight fast, glucagon may provoke no change in blood glucose level. Serum creatine kinase levels can be useful to identify patients with muscle involvement, although normal levels do not rule out muscle enzyme deficiency.

Diagnosis

The histologic appearance of the liver is characterized by a universal distention of hepatocytes by glycogen and the presence of fibrous septa. The fibrosis and the paucity of fat distinguish type III glycogenosis from type I. The fibrosis, which ranges from minimal periportal fibrosis to micronodular cirrhosis, appears in most cases to be progressive. Overt cirrhosis has been seen in some patients with GSD III.

Patients with myopathy and liver symptoms have a generalized enzyme defect (type IIIa). The deficient enzyme activity can be demonstrated not only in liver and muscle but also in other tissues such as heart, erythrocytes, and cultured fibroblasts. Patients with hepatic symptoms without clinical or laboratory evidence of myopathy have debranching enzyme deficiency only in the liver, with enzyme activity retained in the muscle (type IIIb). Before the availability of genetic testing, a definitive diagnosis required enzyme assay in liver, muscle, or both. Gene sequencing allows for diagnosis and subtype assignment in the majority of patients.

Treatment

The mainstay of treatment of GSD III is dietary management, as in GSD I, although it is less demanding. Patients do not need to restrict dietary intake of fructose and galactose, although simple sugars should be avoided to prevent sudden spikes in blood glucose levels. Hypoglycemia can be prevented by small frequent meals rich in complex carbohydrates, such as cornstarch supplements or nocturnal gastric drip feedings. Additionally, a high-protein diet during the daytime as well as overnight protein enteral infusion is effective in preventing hypoglycemia. The exogenous protein can be used as a substrate for gluconeogenesis, which helps to meet energy needs and prevent endogenous

protein breakdown. Protein in the diet also reduces the overall starch requirement. Overtreatment with cornstarch should be avoided, as it can result in excessive glycogen buildup, which is detrimental and can lead to excessive weight gain. Medium-chain triglyceride (MCT) supplementation and a high-fat diet are being considered as alternative sources of energy. There is no satisfactory treatment for the progressive myopathy other than recommending a high-protein diet, physical therapy, and high-fat diet in some patients. Close monitoring with abdominal MRI is needed to detect progression of liver fibrosis to cirrhosis and hepatocellular carcinoma (HCC). Additional imaging techniques, such as hepatic elastography, are being developed. Liver transplantation has been performed in GSD III patients with progressive cirrhosis and/or HCC. There are reports of cardiac transplant in GSD III patients with end-stage cardiac disease.

Type IV Glycogen Storage Disease (Branching Enzyme Deficiency, Amylopectinosis, Polyglucosan Disease, Andersen Disease, Adult Polyglucosan Body Disease)

Type IV GSD is caused by the deficiency of **branching enzyme activity**, which results in the accumulation of an abnormal glycogen with poor solubility. The disease is also known as *amylopectinosis* because the abnormal glycogen has fewer α-1,6 branch points, more α-1,4 linked glucose units, and longer outer chains, resulting in a structure resembling amylopectin. These excessively long peripheral glucan chains form polyglucosan bodies, which are positive on periodic acid–Schiff (PAS) and resistant to diastase digestion. Polyglucosan body accumulation is seen in all affected patients but to different degrees and in different tissues.

Type IV GSD is an autosomal recessive disorder. The glycogen branching enzyme *(GBE1)* gene has been noted, with more than 128 pathogenic variants responsible for type IV GSD, and their characterization in individual patients can be useful in predicting clinical outcome. The nearly complete absence of glycogen branching enzyme (GBE) activity with null alleles has been associated with perinatal death and fatal neonatal hypotonia. Residual GBE activity results in a continuum of disease manifestations ranging from progressive hepatic cirrhosis, slowly progressive or nonprogressive liver disease, cardiomyopathy, myopathy, peripheral neuropathy, motor neuron disease, and leukodystrophy.

Clinical Manifestations

A high degree of clinical variability is associated with type IV GSD. The most common and classic form is characterized by **hepatic** involvement with progressive cirrhosis of the liver and manifests in the first 18 months of life as hepatosplenomegaly and failure to thrive. Cirrhosis may present with portal hypertension, ascites, and esophageal varices and may progress to liver failure, usually leading to death by 5 years of age. Some patients survive without progression of liver disease, and they are considered to have a milder hepatic form and do not require liver transplant. Extrahepatic involvement in some patients with GSD IV consists of musculoskeletal involvement, particularly cardiac and skeletal muscles, as well as central nervous system (CNS) and peripheral nervous system (PNS) involvement.

Neuromuscular forms of type IV GSD have been reported, with four main subtypes recognized based on age at presentation. The **perinatal** form is characterized by a *fetal akinesia deformation sequence* (FADS) and death in the perinatal period. The **congenital** form presents at birth with severe hypotonia, muscle atrophy, and neuronal involvement, with death in the neonatal period; some patients have cardiomyopathy. The **childhood** form presents primarily with myopathy or cardiomyopathy. The **adult** form, *adult polyglucosan body disease* (APBD), presents with PNS dysfunction and diffuse CNS involvement, accompanied by accumulation of polyglucosan material in the nervous system. Symptoms of neuronal involvement include progressive muscle weakness; gait spasticity; peripheral neuropathy; neuropathic bladder; and, in many cases, leukodystrophy, mood disturbances, and cognitive decline. APBD is often misdiagnosed as multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), or cerebral small-vessel disease (CSVD).

Diagnosis

Deposition of amylopectin-like materials varies and can be demonstrated in the liver, heart, muscle, skin, intestine, brain, brainstem, spinal cord, and peripheral nerves (i.e., sural nerve) in type IV GSD. For patients with hepatic involvement, liver histology shows micronodular cirrhosis and faintly stained basophilic inclusions in hepatocytes. The inclusions are composed of coarsely clumped, stored material that is PAS positive and partially resistant to diastase digestion. Electron microscopy (EM) shows, in addition to the conventional α and β glycogen particles, the accumulation of fibrillar aggregations that are typical of amylopectin. Similar nonmembrane-bound cytoplasmic inclusions (PAS positive, diastase resistant) are observed in the cardiomyocytes and skeletal muscle fibers, and polyglucosan bodies may be present in the muscle biopsy from those with neuromuscular manifestations. In APBD, a peripheral nerve biopsy reveals intra-axonal polyglucosan bodies, and histologic examination of brain tissue shows polyglucosan bodies often visualized in the astrocytes and neurons. The distinct staining properties of the inclusions, as well as EM findings, could be diagnostic. However, polysaccharides with histologic features reminiscent of type IV disease but without enzymatic correlation have been observed. The diagnosis is confirmed by either identification of two pathogenic variants in *GBE1* or a reduction of GBE activity in leukocytes or fibroblasts. Prenatal diagnosis is possible by measuring enzyme activity in cultured amniocytes or chorionic villi or by DNA-based methodologies.

Treatment

There is no specific treatment for type IV GSD. Nervous system involvement, such as gait problems and bladder involvement, requires supportive, symptomatic management. Unlike patients with other liver GSDs (I, III, VI, IX), those with GSD IV do not typically have hypoglycemia. Liver transplantation has been performed for patients with progressive liver disease, but patients must be carefully selected because this is a multisystem disease and, in some patients, extrahepatic involvement may manifest or worsen after transplant. Liver transplantation corrects the hepatic phenotype of the disease but has no effects on the extrahepatic manifestations such as myopathy and cardiomyopathy. Individuals with significant diffuse reticuloendothelial involvement may have greater risk of morbidity and mortality, which may affect the success rate for liver transplant. Patients with symptomatic cardiomyopathy and skeletal myopathy may benefit from heart transplantation and physical therapy, respectively.

Type VI Glycogen Storage Disease (Liver Phosphorylase Deficiency, Hers Disease)

Type VI GSD is caused by deficiency of **liver glycogen phosphorylase**. Patients usually present with hepatomegaly and growth stunting in infancy or early childhood. Hypoglycemia, hyperlipidemia, and hyperketosis are of variable severity. Ketotic hypoglycemia may present after overnight or prolonged fasting. Lactic acid and uric acid levels are normal. Type VI GSD presents within a broad spectrum of involvement, some with a more severe clinical presentation. Patients with severe hepatomegaly, recurrent severe hypoglycemia and hyperketosis have been reported. Focal nodular hyperplasia of liver, hepatocellular adenoma with transformation into carcinoma, hepatic fibrosis, and cirrhosis have been reported in some patients.

Diagnosis

GSD VI is an autosomal recessive disease. Diagnosis can be confirmed through molecular testing of the liver phosphorylase gene *(PYGL)*. Many pathogenic variants are known in this gene, and a splice-site variant in intron 13 has been identified in the Mennonite population. A liver biopsy showing elevated glycogen content and decreased hepatic phosphorylase enzyme activity can also be used to make a diagnosis, especially in cases with inconclusive genetic results. However, with the availability of DNA analysis and next-generation sequencing (NGS) panels, liver biopsies are usually unnecessary.

Treatment

Treatment is symptomatic and aimed at preventing hypoglycemia while ensuring adequate nutrition. A low-carbohydrate (45–50% of total calories), high-protein (2–3 g protein/kg body weight or 20–25% of total calories) diet and frequent feeding are effective in preventing hypoglycemia. Blood glucose and ketones should be monitored routinely, especially during periods of increased activity/illness. Long-term follow-up of these patients is needed to monitor and manage the complications and to expand the understanding of the natural history of this disorder.

Type IX Glycogen Storage Disease (Phosphorylase Kinase Deficiency)

Type IX GSD represents a heterogeneous group of glycogenoses resulting from deficiency of the enzyme **phosphorylase kinase** (PhK), which is involved in the rate-limiting step of glycogenolysis. This enzyme has four subunits (α, β, γ, δ), each encoded by different genes differentially expressed in various tissues. Pathogenic variants in the *PHKA1* gene cause muscle PhK deficiency; pathogenic variants in the *PHKA2* and *PHKG2* genes cause liver PhK deficiency, and pathogenic variants in the *PHKB* gene cause PhK deficiency in liver and muscle. Pathogenic variants in the *PHKG1* gene have not been identified.

Clinical manifestations of liver PhK deficiency are usually recognizable within the first 2 years of life and include short stature and abdominal distention from moderate to marked hepatomegaly. The clinical severity of liver PhK deficiency varies considerably. Hyperketotic hypoglycemia, if present, can be mild but may be severe in some cases. Ketosis may occur even when glucose levels are normal. Some children may have mild delays in gross motor development and hypotonia. It is becoming increasingly clear that GSD IX is not a benign condition. Severe phenotypes are reported, with liver fibrosis progressing to cirrhosis and HCC, particularly in patients with *PHKG2* variants and also in some patients with *PHKA2* variants. Progressive splenomegaly and portal hypertension are reported secondary to cirrhosis. Interventricular septal hypertrophy has been reported in a patient with GSD IX β (*PHKB* variant). Cognitive and speech delays have been reported in a few individuals, but it is not clear whether these delays are caused by PhK deficiency or are coincidental. Renal tubular acidosis has been reported in rare cases. Unlike in GSD I, lactic acidosis, bleeding tendency, and loose bowel movements are not characteristic. Although growth is retarded during childhood, normal height and complete sexual development are eventually achieved. As with debrancher deficiency, abdominal distention and hepatomegaly usually decrease with age. Some adults with liver PhK deficiency are reportedly asymptomatic, although patients at the other end of the spectrum develop cirrhosis and liver failure. Further studies are needed to fully assess and understand the natural history of this disorder in adults.

Phenotypic variability within each subtype is being uncovered with the availability of molecular testing. The incidence of all subtypes of PhK deficiency is approximately 1:100,000 live births.

Type IX α2 Glycogen Storage Disease (X-Linked Liver Phosphorylase Kinase Deficiency, PHKA2 Variant)

Glycogen storage disease IX α2 is one of the most common forms of liver glycogenosis in males. A typical presentation for this condition includes a young male (age 1-5 years) with growth stunting, incidental finding of hepatomegaly, and mild delays in motor development. In addition to the liver, enzyme activity can also be deficient in erythrocytes, leukocytes, and fibroblasts, with normal enzyme activity in muscle. Cholesterol, triglycerides, and liver enzymes are elevated. Ketosis may occur after fasting. Lactate and uric acid levels are normal. Hypoglycemia can range from mild to severe. The response in blood glucose to glucagon is normal. Although hepatomegaly and liver enzymes might gradually reduce and normalize with age in some patients, other groups exhibit an increase in these disease parameters comparable to those observed in patients with GSD IX γ 2. Most adults achieve a normal final height. It is increasingly recognized that this disorder is not benign, as previously thought, and there are patients with severe disease and long-term hepatic sequelae. In some cases, liver fibrosis can occur and progress to cirrhosis. Liver histology shows glycogen-distended hepatocytes, steatosis, fibrosis, and/or cirrhosis. Fibrous septal formation and low-grade inflammatory changes may be seen. Adenomas have been also reported.

The gene for the common liver isoform of the PhK α subunit, *PHKA2,* is located on the X chromosome (Xp22.2). Pathogenic variants in the *PHKA2* gene account for 75% of all PhK cases. X-linked liver PhK deficiency is further subdivided into two biochemical subtypes: XLG1, with measurable deficiency of PhK activity in both blood cells and liver, and XLG2, with normal in vitro PhK activity in blood cells and variable activity in the liver. The mechanism through which XLG2 exhibits normal or increased in vitro PhK activity is unknown. Because of X-inactivation leading to variable expression of the mutant allele, some female carriers may develop symptoms ranging from mild hepatomegaly to more severe symptoms.

Type IX β Glycogen Storage Disease (Autosomal Liver and Muscle Phosphorylase Kinase Deficiency, PHKB Variant)

PhK deficiency in liver and blood cells with an autosomal recessive mode of inheritance has been reported. Similar to the X-linked form, chief symptoms in early childhood include hepatomegaly and growth retardation. Some patients also exhibit muscle hypotonia. In a few cases where enzyme activity has been measured, reduced PhK activity has been demonstrated in muscle. Pathogenic variants are found in PHKB (chromosome 16q12-q13), which encodes the β subunit, and result in liver and muscle PhK deficiency (GSD IX β). Several nonsense and missense variants, a single-base insertion, splice-site pathogenic variants, and a large intragenic pathogenic variant have been identified.

Type IX γ2 Glycogen Storage Disease (Autosomal Liver Phosphorylase Kinase Deficiency, PHKG2 Variant)

This form of PhK deficiency is caused by pathogenic variants in the testis/liver isoform (TL) of the γ2 subunit of the *PHKG2* gene. In contrast to GSD IX α2, patients with GSD IX γ2 typically have more severe phenotypes, with recurrent hypoglycemia, prominent hepatomegaly, significant liver fibrosis, and progressive cirrhosis. Hepatic adenomas have been observed in several patients. Liver involvement occasionally presents with cholestasis, bile duct proliferation, esophageal varices, and splenomegaly. The spectrum of involvement continues to evolve as more cases are recognized. Many pathogenic variants in the *PHKG2* gene have been identified.

Diagnosis

Individuals with liver PhK deficiency usually have ketotic hypoglycemia, elevated transaminases, elevated triglycerides and cholesterol, normal uric acid and lactic acid concentrations, and normal glucagon response. PhK deficiency may be diagnosed by demonstration of the enzymatic defect in affected tissues. Hepatic PhK activity can be measured in liver, leukocytes, and erythrocytes. The interpretation of PhK activity results is complicated by the possibility of both false-positive and false-negative results. False-positive findings are possible, owing to the labile nature of PhK, which is extremely sensitive to handling circumstances and temperature exposure. As a result, extreme caution is required when storing and transporting such diagnostic specimens. Because the PhK enzyme has several isozymes, the diagnosis can easily be missed without the tissue where it is deficient (liver, muscle, or cardiac studies).

Molecular genetic testing is preferable to enzyme assay for diagnosis, as liver biopsy is an invasive procedure. Gene sequencing is used for diagnostic confirmation and subtyping of GSD IX. The *PHKA2* gene encoding the α subunit is most frequently involved, followed by the *PHKB* and *PHKG2* genes encoding the β and γ subunits, respectively.

Treatment and Prognosis

The treatment for liver PhK deficiency is symptomatic and includes a high-protein (2-3 g protein/kg body weight or 20–25% of total calories) diet with complex carbohydrates (45–50% of total calories) and small, frequent feedings to prevent hypoglycemia. Cornstarch can be administered, with symptom-dependent dosage and timing (0.6-2.5 g/kg every 6 hours). Hypoglycemia should be treated with oral glucose, if tolerated; if not, IV glucose should be given.

Patients with pathogenic variants in the γ subunit typically have a more severe clinical course with progressive liver disease. In liver biopsy samples, hepatic fibrosis/cirrhosis was detected in 50% and 95% of patients with *PHKA2* and *PHKG2* pathogenic variants, respectively. Two patients with GSD IX γ2 received liver transplantation, one for liver failure secondary to cirrhosis and the other for HCC. Liver involvement needs to be monitored in all patients with GSD IX by periodic imaging (abdominal ultrasound or MRI every 6-12 months) and serial hepatic function tests.

Type 0a Glycogen Storage Disease (Liver Glycogen Synthase Deficiency)

Liver glycogen synthase deficiency type 0a **(GSD 0a)** is caused by deficiency of **hepatic glycogen synthase** activity, leading to a marked decrease of glycogen stored in the liver. It is caused by pathogenic variants in *GYS2*. The disease appears to be rare in humans and, in the true sense, is not a type of GSD because the enzyme deficiency leads to decreased glycogen stores. Patients present in infancy with early-morning (pre-breakfast) drowsiness, pallor, emesis, and fatigue, and occasional convulsions associated with hypoglycemia and hyperketonemia. Blood lactate and alanine levels are low, and there is no hyperlipidemia or hepatomegaly. **Hyperglycemia**, glycosuria, lactic acidosis, and hyperalaninemia, with normal insulin levels after administration of glucose or a meal, suggest a deficiency of glycogen synthase. ***Diagnosis*** may be through molecular testing that identifies pathogenic variants in *GYS2* or by liver biopsy to measure the enzyme activity.

Treatment consists of frequent meals rich in protein and nighttime supplementation with uncooked cornstarch to prevent hypoglycemia and hyperketonemia. Most children with GSD 0a are cognitively and developmentally age appropriate. Short stature and osteopenia are common features. The ***prognosis*** seems good for patients who survive to adulthood, with a decrease in the frequency and severity of hypoglycemia. Long-term natural history studies are required to have a better understanding of the disease's outcomes and the impact of dietary therapy.

Hepatic Glycogenosis with Renal Fanconi Syndrome (Fanconi-Bickel Syndrome)

Fanconi-Bickel syndrome is a rare autosomal recessive disorder caused by defects in the facilitative GLUT2, which transports glucose in and out of hepatocytes, pancreatic β cells, and the basolateral membranes of intestinal and renal epithelial cells. The disease is characterized by proximal renal tubular dysfunction, impaired glucose and galactose utilization, and accumulation of glycogen in the liver and kidney.

The affected child typically presents in the first year of life with failure to thrive, rickets, and a protuberant abdomen from hepatomegaly and nephromegaly. The disease may be confused with GSD I because a Fanconi-like syndrome can also develop in that patient population. Adult patients have short stature as a result of hypophosphatemic rickets. Patients are more susceptible to fractures because of early-onset generalized osteopenia. In addition, intestinal malabsorption and diarrhea may occur, and HCC has been seen. Laboratory findings include glucosuria, phosphaturia, generalized aminoaciduria, bicarbonate wasting, hypophosphatemia, increased serum alkaline phosphatase levels, and radiologic findings of rickets. Mild fasting hypoglycemia and hyperlipidemia may be present. Liver transaminase, plasma lactate, and uric acid levels are usually normal. Oral galactose or glucose tolerance tests show intolerance, which could be explained by the functional loss of GLUT2 preventing liver uptake of these sugars. Tissue biopsy results show marked accumulation of glycogen in hepatocytes and proximal renal tubular cells, presumably from the altered glucose transport out of these organs. Diffuse glomerular mesangial expansion, along with glomerular hyperfiltration and microalbuminuria similar to nephropathy in GSD Ia, and diabetes have been reported.

This condition is rare, and 70% of patients with Fanconi-Bickel syndrome have consanguineous parents. Most patients have homozygous pathogenic variants; some patients are compound heterozygotes. Several types of pathogenic variants in the *SLC2A2* gene have been described, including missense, nonsense, insertion/deletion, and intronic variants.

There is no specific treatment. Symptom-dependent treatment with phosphate, bicarbonate, and uncooked cornstarch can result in growth improvement. Growth may also improve with symptomatic replacement of water, electrolytes, and vitamin D and restriction of galactose intake; a diet similar to that used for diabetes mellitus, with small, frequent meals using complex carbohydrates and adequate caloric intake, is recommended.

MUSCLE GLYCOGENOSES

The role of glycogen in muscle is to provide substrates for the generation of ATP for muscle contraction. The muscle GSDs are broadly divided into two groups. The first group is characterized by hypertrophic cardiomyopathy, progressive skeletal muscle weakness and atrophy, or both and includes deficiencies of acid α-glucosidase, a lysosomal glycogen-degrading enzyme (**type II** GSD); lysosomal-associated membrane protein 2 **(LAMP2/Danon disease)**; AMP-activated protein kinase γ2 **(PRKAG2/PRKAG2 syndrome)**; and muscle glycogen synthase **(type 0b)**. Glycogenin-1 deficiency **(GYG1/polyglucosan body myopathy 2)** may manifest as muscle weakness and exercise intolerance to exercise in the absence of cardiomyopathy.

The second group comprises muscle energy disorders characterized by muscle pain, exercise intolerance, myoglobinuria, and susceptibility to fatigue. This group includes myophosphorylase deficiency (McArdle disease, **type V** GSD) and deficiencies of phosphofructokinase **(type VII)**, phosphoglycerate kinase, phosphoglycerate mutase, lactate dehydrogenase (LDH), and muscle-specific phosphorylase kinase (**type IX** α1). Some of these latter enzyme deficiencies can also be associated with *compensated hemolysis,* suggesting a more generalized defect in glucose metabolism.

Type II Glycogen Storage Disease (Lysosomal Acid α-1,4-Glucosidase Deficiency, Pompe Disease)

Pompe disease, also referred to as *GSD type II* or **acid maltase deficiency**, is caused by a deficiency of acid α-1,4-glucosidase, an enzyme responsible for the degradation of glycogen in lysosomes. This enzyme defect results in lysosomal glycogen accumulation in multiple tissues and cell types, predominantly affecting cardiac, skeletal, and smooth muscle cells and the nervous system. In Pompe disease, glycogen typically accumulates within lysosomes, as opposed to its accumulation in cytoplasm in the other glycogenoses. However, as the disease progresses, lysosomal rupture and leakage lead to the presence of cytoplasmic glycogen as well.

Pompe disease is an autosomal recessive disorder. The incidence was previously reported to be approximately 1 in 40,000 live births in Whites and 1 in 18,000 live births in the Han Chinese population. Newborn screening for Pompe disease in the United States suggests that the prevalence is much higher than previously thought (between 1 in 10,000 and 1 in 25,000). More than 600 pathogenic variants have been identified in the gene for acid α-glucosidase *(GAA)* and are helpful in delineating the phenotypes. A splice-site variant (c.-32-13 T > G, formerly called IVS1-13 T > G) is commonly seen in White patients with late-onset disease.

Clinical Manifestations

Pompe disease is broadly classified into infantile and late-onset forms. The infantile presentation is a continuum and is further divided into two categories: classic and nonclassic. Classic **infantile Pompe disease** (IPD) is uniformly lethal *without* enzyme replacement therapy (ERT) with *alglucosidase alfa*. Affected infants present in the first days to weeks of life with hypotonia, generalized muscle weakness with a hypotonic *floppy infant* appearance, hypertrophic cardiomyopathy

with or without left ventricular outflow obstruction, feeding difficulties, macroglossia, and hepatomegaly; if untreated, the condition leads to death from cardiorespiratory failure or respiratory infection by 1 year of age. Nonclassic IPD has a slower disease course and a less severe cardiac phenotype at initial presentation.

Late-onset Pompe disease (LOPD; juvenile-, childhood-, and adult-onset disease) is characterized by proximal limb girdle muscle weakness and early involvement of respiratory muscles, especially the diaphragm. The distinguishing feature between IPD and LOPD is absence of cardiomyopathy in the first year of life in patients with LOPD. Symptoms related to progressive dysfunction of skeletal muscles can start within the first year of life to as late as the sixth decade. The clinical picture is dominated by slowly progressive proximal muscle weakness, with truncal involvement and greater involvement of the lower limbs than the upper limbs. The pelvic girdle, paraspinal muscles, and diaphragm are the muscle groups most seriously affected in patients with LOPD. Cardiac involvement can occur and ranges from cardiac rhythm disturbances to cardiomyopathy. Other symptoms may include lingual weakness, ptosis, and dilation of blood vessels (e.g., basilar artery, ascending aorta). With disease progression, patients become confined to a wheelchair and require artificial ventilation. The initial symptoms in some patients may be respiratory insufficiency manifested by somnolence, morning headache, orthopnea, and exertional dyspnea, which eventually lead to sleep-disordered breathing and respiratory failure. Respiratory failure is the cause of significant morbidity and mortality in LOPD. Basilar artery aneurysms with rupture also contribute to mortality in some cases. Small-fiber neuropathy presenting as painful paresthesia has been identified in some LOPD patients. Gastrointestinal disturbances such as postprandial bloating, dysphagia, early satiety, diarrhea, chronic constipation, and IBD have been reported. Genitourinary tract involvement is not uncommon and may present as bladder and bowel incontinence, weak urine stream, or dribbling. If untreated, the age of death varies from early childhood to late adulthood, depending on the rate of disease progression and the extent of respiratory muscle involvement. With the advent of ERT, a new natural history is emerging for survivors of both IPD and LOPD.

Laboratory Findings

Pertinent laboratory findings include elevated levels of serum creatine kinase (CK), aspartate transaminase (AST), alanine transaminase (ALT), and LDH. Urine glucose tetrasaccharide (Glc4), a glycogen breakdown metabolite, is a reliable biomarker for gauging disease severity and progression, as well as treatment response. Levels of Glc4 are extremely elevated in patients with IPD. In the infantile form, a chest x-ray film showing massive cardiomegaly is frequently the first symptom detected. **Electrocardiographic findings** include a high-voltage QRS complex, Wolff-Parkinson-White (WPW) syndrome, and a *shortened* PR interval. **Echocardiography** reveals thickening of both ventricles and/or the intraventricular septum and/or left ventricular outflow tract obstruction. Dilated cardiomyopathy and a low ejection fraction have also been reported. Muscle biopsy shows the presence of vacuoles that stain positively for glycogen; acid phosphatase is increased, presumably from a compensatory increase of lysosomal enzymes. EM reveals glycogen accumulation within a membranous sac and with disease progression also in the cytoplasm. EMG reveals myopathic features with excessive electrical irritability of muscle fibers and pseudomyotonic discharges. Serum CK is not always elevated in adult patients. Depending on the muscle sampled or tested, the muscle histologic appearance and electromyography may not be abnormal. Some patients with infantile Pompe disease who had peripheral nerve biopsies demonstrated glycogen accumulation in the neurons and Schwann cells.

Diagnosis

A diagnosis of Pompe disease can be made by either enzyme assay in dried blood spots, leukocytes, blood mononuclear cells, muscle, or cultured skin fibroblasts demonstrating deficient acid α-glucosidase activity or gene sequencing showing two pathogenic variants in the *GAA* gene. The enzyme assay should be done in a laboratory with experience using maltose, glycogen, or 4-methylumbelliferyl-α-d-glucopyranoside (4MUG) as a substrate. In fibroblast enzyme assays, the infantile form has a more severe enzyme deficiency (less than 1% of that in normal controls) than the late-onset forms (between 1% and 30% of that in normal controls). Blood-based assays, especially dried blood spots, have the advantage of being quick and noninvasive and are increasingly being used as the first-line sample to make a diagnosis. The presence of the neutral α-glucosidase isoenzyme, which interferes with acid glucosidase, was formerly considered to be a disadvantage in blood-based assays. However, the addition of an inhibitor to this isozyme, acarbose, improves the assay's reliability by blocking isoenzyme activity. A muscle biopsy is often done with suspected muscle disease and a broad diagnostic differential, as it yields faster results and provides additional information about glycogen content and site of glycogen storage within and outside the lysosomes of muscle cells. However, a normal muscle biopsy, especially in patients with LOPD, *does not* exclude a diagnosis of Pompe disease. Late-onset patients show variability in glycogen accumulation in different muscles and within muscle fibers, and muscle histology and glycogen content can vary depending on the site of muscle biopsy. Because of the high risk of complications, anesthesia in infantile cases with significant cardiomyopathy should be reserved for situations where it is necessary. Availability of NGS panels and whole exome or genome sequencing allow for identification of additional patients with Pompe disease, especially when the diagnosis is ambiguous. *GAA* enzyme activity can be measured in chorionic villi or amniocytes for prenatal diagnosis; however, if the familial pathogenic variants are known, molecular genetic testing is the recommended approach.

Treatment

ERT with recombinant human acid α-glucosidase (alglucosidase alfa and avalgluco) is available for treatment of Pompe disease. Recombinant acid α-glucosidase can prevent deterioration or reversing abnormal cardiac and skeletal muscle functions (Fig. 107.3). ERT should be initiated as soon as possible across the disease spectrum, especially for infants with the infantile form, because the disease is rapidly progressive. Infants who are negative for cross-reacting immunologic material (CRIM) (i.e., infants that have no detectable GAA on a Western blot), develop a high-titer antibody against the infused enzyme and respond to ERT less favorably. A subset of CRIM-positive patients (presence of some protein on a Western blot) can also develop high and sustained antibody titers to ERT. Treatment using immunomodulating agents such as methotrexate, rituximab, and intravenous immunoglobulin (IVIG) have demonstrated efficacy in preventing the development of an immune response to ERT and immune tolerance. Nocturnal ventilatory support, when indicated, should be used. It has been shown to improve the quality of life and is particularly beneficial during a period of respiratory decompensation. In addition to ERT, other **adjunctive therapies** have demonstrated benefit in patients with Pompe disease. For patients with late-onset disease, a high-protein, low-carbohydrate diet may be beneficial. Respiratory muscle strength training has demonstrated improvements in respiratory parameters when combined with ERT. Submaximal aerobic exercise regimens are beneficial in improving muscle strength, pain, and fatigue.

A second-generation ERT, **avalglucosidase alfa** (NeoGAA), has been recently approved by the Food and Drug Administration (FDA) for the treatment of individuals with LOPD older than 1 year of age. Mannose-6-phosphate (M6P) is more abundant in NeoGAA than in alglucosidase alfa, which improves enzyme uptake into cells. Improvements in respiratory function (forced vital capacity [FVC]) and 6-minute walk test (6MWT) in patients with LOPD have been shown by phase 1/2 and 3 trials.

There are other therapies in development for Pompe disease, including intravenous **cipaglucosidase alfa** (a novel recombinant human GAA with high bis-M6P content; functions as substrate reduction therapy), **miglustat** (an oral chaperone; functions as enzyme stabilizer), small-molecule therapy, and gene therapy.

Early diagnosis and treatment are necessary for optimal outcomes. Newborn screening using blood-based assays in Taiwan and 28 U.S. states has resulted in early identification of Pompe cases, and thus improved disease outcomes, through the early initiation of ERT.

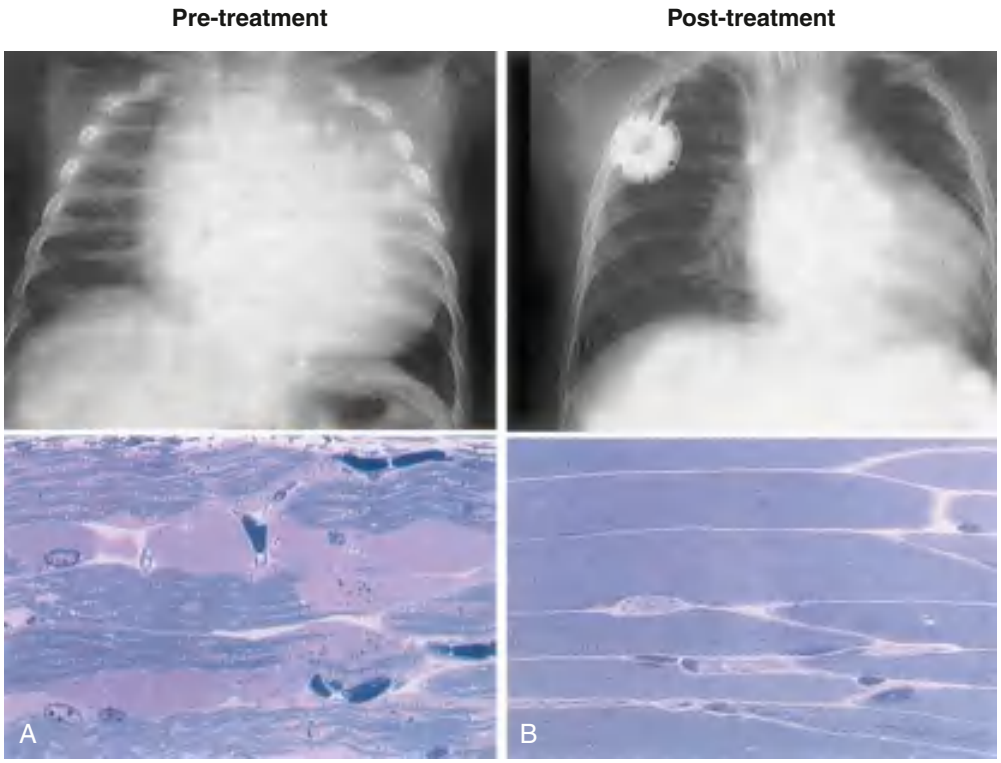

Fig. 107.3 Chest radiograph and muscle histology findings of an infantile-onset Pompe disease patient before (A) and after (B) enzyme replacement therapy. Note the decrease in heart size and muscle glycogen with therapy. *(Modified from Amalfitano A, Bengur AR, Morse RP, et al. Recombinant human acid alpha-glucosidase enzyme therapy for infantile glycogen storage disease type II: results of a phase I/II clinical trial. Genet Med. 2001;3:132–138.)*

Danon Disease

Danon disease is caused by pathogenic variants in the *LAMP2* gene, which leads to a deficiency of **lysosomal-associated membrane protein 2** (LAMP2). This disorder is inherited in an X-linked dominant pattern. Defects in LAMP2 lead to accumulation of glycogen in the heart and skeletal muscle, presenting primarily as hypertrophic cardiomyopathy and skeletal muscle weakness. Danon disease can be distinguished from the usual causes of hypertrophic cardiomyopathy (defects in sarcomere-protein genes) by the electrophysiologic abnormalities, particularly ventricular preexcitation and conduction defects. Patients present with cardiac symptoms, including chest pain, palpitations, syncope, and cardiac arrest, usually between ages 8 and 15 years. Other clinical manifestations include peripheral pigmentary retinopathy, lenticular changes, abnormal electroretinogram, and mild cognitive dysfunction. ***Diagnosis*** can be established by molecular testing of the *LAMP2* gene. The prognosis for LAMP2 deficiency is poor, with progressive end-stage heart failure early in adulthood. ***Treatment*** is directed toward management of symptoms in affected individuals, including management of cardiomyopathy, correction of arrhythmias, and physical therapy for muscle weakness. Cardiac transplantation has been successful in some patients. In male patients with Danon disease, a nonrandomized open-label phase 1 study (NCT03882437) is currently being conducted to assess the safety of gene therapy using recombinant adeno-associated virus serotype 9 (AAV9) containing the human lysosome-associated membrane protein 2 isoform B (LAMP2B) transgene.

PRKAG2 Syndrome (Adenosine Monophosphate [AMP]–Activated Protein Kinase γ2 Deficiency)

PRKAG2 syndrome is caused by pathogenic variants in the *PRKAG2* gene that is required for the synthesis of the enzyme AMP-activated protein kinase (AMPK), which regulates cellular pathways involved in ATP metabolism. PRKAG2 syndrome has an autosomal dominant pattern of inheritance. Common presentations include hypertrophic cardiomyopathy and electrophysiologic abnormalities such as WPW syndrome, atrial fibrillation, and progressive atrioventricular block. Cardiac involvement is variable and includes supraventricular tachycardia, sinus bradycardia, left ventricular dysfunction, and sudden cardiac death in some cases. In addition to cardiac involvement, there is a broad spectrum of phenotypic presentations including myalgia, myopathy, and seizures. Cardiomyopathy caused by *PRKAG2* variants usually allows for long-term survival, although a rare congenital form presenting in early infancy is associated with a rapidly fatal course. Cardiomyopathy in PRKAG2 syndrome often mimics that in other conditions, especially Pompe disease, and should be considered as a differential diagnosis in infants presenting with severe hypertrophic cardiomyopathy. ***Treatment*** is primarily symptomatic, including management of cardiac failure and correction of conduction defects. Patients with PRKAG2 deficiency are at risk for sudden cardiac death and require close monitoring.

Type 0b Glycogen Storage Disease (Muscle Glycogen Synthase Deficiency)

Muscle glycogen synthase deficiency (GSD 0b) results from biallelic loss-of-function pathogenic variants in the gene *GYS1*. In the true sense, this is not a type of GSD because the enzyme deficiency leads to decreased glycogen stores in skeletal and cardiac muscles. The disease is extremely rare and has only been reported in five cases. Muscle biopsies showed lack of glycogen, predominantly oxidative fibers, and mitochondrial proliferation. Glucose tolerance testing was normal. The phenotype was variable and ranged from sudden cardiac arrest, muscle fatigability, hypertrophic cardiomyopathy, abnormal heart rate, and hypotension while exercising to mildly impaired cardiac function at rest.

Type XV Glycogen Storage Disease (Glycogenin-1 Deficiency, Polyglucosan Body Myopathy 2, GYG1 Variant)

Polyglucosan body myopathy 2 is an autosomal recessive, slowly progressive skeletal myopathy caused by pathogenic variants in the *GYG1*

gene disrupting **glycogenin-1** biosynthesis. There is a reduced or complete absence of glyogenin-1, which is a precursor necessary for glycogen formation. Polyglucosan accumulation in skeletal muscles causes juvenile- or adult-onset proximal muscle weakness, prominently affecting the hip and shoulder girdles. Some patients were noted to have a cardiac phenotype. Exertion-induced chest pain, palpitations, and shortness of breath are common early symptoms. Changes in electrophysiological (ECG) parameters and impairment of left ventricular function were reported. Heart transplantation may be necessary in some cases. The missense variant c.304G>C (p. Asp102His) has been observed in homozygosity in the majority of these patients. Compared with GSD IV–APBD, nervous system involvement is uncommon, although polyglucosan deposition is seen in both disorders. Muscle biopsies show PAS-positive, diastase-resistant storage material in 30–40% of muscle fibers. EM reveals the typical polyglucosan structure, consisting of an ovoid form composed of partly filamentous material.

Type V Glycogen Storage Disease (Muscle Phosphorylase Deficiency, McArdle Disease)

GSD type V, the prototype of muscle energy disorders, is one of the most common GSDs with a prevalence of ~1 in 10,000 and is caused by deficiency of **myophosphorylase** activity. Lack of this enzyme limits muscle ATP generation from glycogenolysis, resulting in muscle glycogen accumulation. A deficiency of myophosphorylase impairs the cleavage of glucosyl molecules from the straight chain of glycogen.

Clinical Manifestations

Symptoms usually first develop in late childhood or in the second decade of life. Studies have shown that McArdle disease can manifest in individuals as old as in their seventh decade and in infancy in a fatal, early-onset form characterized by hypotonia, generalized muscle weakness, and respiratory complications. Symptoms are generally characterized by exercise intolerance with muscle cramps and pain and are precipitated by two types of activity: (1) brief, high-intensity exercise, such as sprinting or carrying heavy loads and (2) less intense but sustained activity, such as climbing stairs or walking uphill. Most patients can perform moderate exercise, such as walking on level ground, for long periods. Many patients experience a characteristic *second-wind phenomenon,* with relief of muscle pain and fatigue after a brief period of rest. An increased blood flow of glucose derived from either endogenous liver glycogenolysis or exogenous glucose and free fatty acids, which may be used as an alternative energy source by the exercising muscles, causes the second-wind phenomenon. As a result of the underlying myopathy, these patients may be at risk for **statin-induced myopathy** and rhabdomyolysis. Although patients typically experience episodic muscle pain and cramping from exercise, up to 35% of patients with McArdle disease report permanent pain that has a serious impact on sleep and other activities.

Approximately 50% of patients report burgundy-colored urine after exercise as a result of exercise-induced **myoglobinuria** secondary to **rhabdomyolysis**. Excessive myoglobinuria after intense exercise may precipitate acute renal failure.

Laboratory findings include elevated serum CK levels at rest, which further increase after exercise. Exercise also elevates the levels of blood ammonia, inosine, hypoxanthine, and uric acid, which may be attributed to accelerated recycling of muscle purine nucleotides caused by insufficient ATP production.

Diagnosis

Type V GSD is an autosomal recessive disorder. Testing of the gene for muscle phosphorylase *(PYGM)* helps confirm the diagnosis of GSD V. A common nonsense variant, c.148C>T (p.Arg50Ter) in exon 1, is found in 80% of White patients; deletion of a single codon, c.2128_2130del (p.Phe710del) in exon 17, is found in 61% of patients of Japanese origin. The c.148C>T (p.Arg50Ter) variant represents 55% of alleles in patients of Spanish descent, and the c.2392T>C (p.Trp798Arg) and c.613G>A (p.Gly205Ser) variants represent 10% and 9% of pathogenic alleles in this population. Muscle biopsy can be used to measure glycogen content and enzyme activity in cases where genetic testing results are inconclusive. Lack of an increase in blood lactate levels and exaggerated blood ammonia elevations suggest a defect in the conversion of muscle glycogen or glucose to lactate. An ischemic exercise test offers rapid diagnostic screening for patients suspected to have McArdle disease, though it should be noted that an abnormal response is *not limited* to type V GSD, as other muscle defects in glycogenolysis or glycolysis produce similar results (deficiencies of muscle phosphofructokinase, phosphoglycerate kinase, phosphoglycerate mutase, or LDH). Additionally, such testing was also associated with severe complications and false-positive results. A nonischemic forearm exercise test with high sensitivity that is easy to perform and is cost-effective has been deemed a more appropriate diagnostic tool; however, it also cannot differentiate abnormal exercise responses due to type V disease from other defects in glycogenolysis or glycolysis.

Treatment

To enhance patients' outcomes and physical activity capacity, a multidisciplinary team approach to care composed of a physician, physiotherapist, psychologist, clinical nurse, and dietitian is advised. Avoidance of strenuous exercise prevents symptoms; regular and moderate exercise is recommended to improve exercise capacity. Patients are advised to take advantage of the second-wind phenomenon by commencing exercise slowly and to slow down or stop if muscle weakness or discomfort, increased heart rate, or increased respiratory effort occurs. This *slow-pause-resume* pattern is intended to be maintained as needed until considerable improvement in physical activity tolerance occurs.

High-dose oral ribose, glucagon, verapamil, vitamin B_6, a high-protein diet, branched-chain amino acid supplementation, dantrolene sodium, high-dose creatine, intravenous gentamicin, and intralipid infusion treatments showed no benefit according to a revised and updated systematic review in the Cochrane Database of nutritional and pharmacologic trials for GSD V. Oral sucrose ingestion before exercise, a carbohydrate-rich diet, ramipril, and low-dose creatine were treatments that showed some promise. There is ongoing research regarding the benefits of a low-carbohydrate ketogenic diet in patients with type V GSD.

Type VII Glycogen Storage Disease (Muscle Phosphofructokinase Deficiency, Tarui Disease)

Type VII GSD is caused by pathogenic variants in the *PFKM* gene, which result in a deficiency of **muscle phosphofructokinase** enzyme. This enzyme is a key regulatory enzyme of glycolysis and is necessary for the ATP-dependent conversion of fructose-6-phosphate to fructose-1,6-diphosphate. Phosphofructokinase is composed of three isoenzyme subunits according to the tissue type, encoded by different genes: (*PFKM* [M: muscle], *PFKL* [L: liver], and *PFKP* [P: platelet]). Skeletal muscle has only the M subunit, whereas red blood cells (RBCs) express a combination of L and M forms. In type VII GSD, the M isoenzyme is defective, resulting in complete deficiency of enzyme activity in muscle and a partial deficiency in RBCs.

Type VII GSD is an autosomal recessive disorder with increased prevalence in individuals of Japanese ancestry and Ashkenazi Jewish background. A splicing defect and a nucleotide deletion in *PFKM* account for 95% of pathogenic variants in the Ashkenazi Jewish population. Diagnosis based on molecular testing for the common variants is possible in this population.

Clinical Manifestations

Although the clinical picture is similar to that of type V GSD, the following features of type VII GSD are distinctive:

- Exercise intolerance, which usually commences in childhood, is more severe than in type V disease and may be associated with nausea, vomiting, and severe muscle pain. Vigorous exercise causes severe muscle cramps and myoglobinuria.
- Compensatory hemolysis occurs, as indicated by an increased level of serum bilirubin and an elevated reticulocyte count.
- Hyperuricemia is common and exaggerated by muscle exercise to a greater degree than that observed in type V GSD.

In addition to normal glycogen, GSD VII is characterized by the accumulation of polyglucosan bodies. The accumulation of glucose-6-phosphate caused by PFKM deficiency and the inhibition of glycolysis stimulate the glycogen synthase enzyme, resulting in the formation of polyglucosan bodies.

Exercise intolerance is especially worse after carbohydrate-rich meals because the ingested glucose prevents lipolysis, thereby depriving muscle of fatty acid and ketone substrates. This is called the *out-of-wind phenomenon,* in contrast to patients with type V disease who can metabolize blood-borne glucose derived from either endogenous liver glycogenolysis or exogenous glucose. Indeed, glucose infusion improves exercise tolerance in patients with type V disease.

The *second-wind phenomenon* is absent because of the inability to break down blood glucose.

Several rare type VII presentations have been described. One form presents in infancy with hypotonia and limb weakness and proceeds to a rapidly progressive myopathy that leads to death by 4 years of age. A second type occurs in infancy and results in congenital myopathy and arthrogryposis, with a fatal outcome. A third form presents in infancy with hypotonia, mild developmental delay, and seizures. An additional presentation is *hereditary nonspherocytic hemolytic anemia;* although these patients do not experience muscle symptoms, it remains unclear whether these symptoms will develop later in life. Another group of individuals with asymptomatic partial red cell PFK deficiency has been described. One phenotype presents in adults and is characterized by a slowly progressive, fixed muscle weakness rather than cramps and myoglobinuria. It may also cause mitral valve thickening from glycogen buildup. Myopathy and hemolysis are hallmarks of the classic form.

Diagnosis

To establish a diagnosis, gene sequencing can identify pathogenic variants in the *PFKM* gene. Demonstration of the enzymatic deficiency in muscle may be required in some cases. The absence of the M isoenzyme of phosphofructokinase can also be demonstrated in muscle, blood cells, and fibroblasts.

Treatment

Strenuous exercise should be avoided to prevent acute episodes of muscle cramps, myoglobinuria, acute renal failure, and compartment syndrome. Continuous blood pressure monitoring or compressive devices, as well as the use of tourniquets, are contraindicated in these patients. Patients with GSD VII should follow the same *slow-pause-resume* routine as those with GSD V. Dietary therapy for GSD VII has not been thoroughly investigated. A ketogenic diet has been reported to show clinical improvement in a patient with GSD VII. Carbohydrate meals and glucose infusions have demonstrated worsening symptoms because of the body's inability to use glucose. The administered glucose tends to lower the levels of fatty acids in the blood—a primary source of muscle fuel. *Drugs such as statins should be avoided.* Precautionary measures should be taken to avoid hyperthermia, hypothermia, hypoglycemia, and shivering while undergoing anesthesia in both GSD V and GSD VII.

Type IX α1 Glycogen Storage Disease (Muscle-Specific Phosphorylase Kinase Deficiency, *PHKA1* Variant)

X-linked muscle PhK deficiency (IX α1 GSD) is caused by pathogenic variants in the *PHKA1* gene, which encodes a muscle-specific regulatory subunit (α subunit) of phosphorylase kinase and is located on Xq13.11. The condition is male-predominant, but affected heterozygous females have been reported. Patients generally present with mild to severe exercise intolerance in childhood or adolescence. Most patients have elevated serum CK and, in more involved cases, myoglobulinuria. There is no evidence of hepatic or cardiac disease in these patients. Blood phosphorylase kinase enzymatic activity is normal, and the enzyme activity is decreased to deficient in the muscle. Muscle biopsy reveals subsarcolemmal accumulations of glycogen, and some patients have myopathic changes on EMG. One patient has been described with comorbid muscle PhK deficiency and intellectual disability; although a connection between *PHKA1* and neurodevelopment has not yet been established, patients with the diagnosis should be monitored for developmental concerns.

The gene for the muscle γ subunit *(PHKG1)* is on chromosome 7p11.2, and no pathogenic variants in this gene have been reported to date.

Other Muscle Glycogenoses with Muscle Energy Impairment

Five additional defects in enzymes—phosphoglycerate kinase (PGK), phosphoglycerate mutase (PGAM), LDH, fructose-1,6-bisphosphate aldolase (aldolase A), and β-enolase in the pathway of the terminal glycolysis—cause symptoms and signs of muscle energy impairment similar to those in types V and VII GSD. Deficiency in PGAM, enolase, or LDH causes a myopathic phenotype marked by exercise-induced cramps and myoglobinuria. Patients with aldolase A or PGK deficiencies may present with hemolytic anemia in conjunction with myopathy. The failure of blood lactate to increase in response to exercise is a useful screening test and can be used to differentiate muscle glycogenoses from disorders of lipid metabolism, such as carnitine palmitoyltransferase II deficiency and very long-chain acyl-CoA dehydrogenase deficiency, which also cause muscle cramps and myoglobinuria. Muscle glycogen levels can be normal in the disorders affecting terminal glycolysis, and molecular testing or muscle enzyme activity assay is needed to make a definitive diagnosis. There is no specific treatment (see preceding "Treatment" section).

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107.2 Defects in Galactose Metabolism

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Milk and dairy products contain **lactose**, the major dietary source of galactose. The metabolism of galactose produces fuel for cellular metabolism through its conversion to glucose-1-phosphate (see Table 107.1). Galactose also plays an important role in the formation of galactosides, which include glycoproteins, glycolipids, and glycosaminoglycans. **Galactosemia** is a term that refers to an abnormally high quantity of galactose in the blood. It can be caused by one of four distinct inborn defects in galactose metabolism involving one of the following enzymes: galactose mutarotase (GALM), galactokinase (GALK), galactose-1-phosphate uridyl transferase (GALT), and uridine diphosphate galactose-4-epimerase (GALE). Among these, the most serious defect is severe GALT deficiency or classic galactosemia, which is frequently referred to as *galactosemia*.

GALACTOSE-1-PHOSPHATE URIDYL TRANSFERASE DEFICIENCY GALACTOSEMIA

Two forms of the deficiency exist: infants with complete or near-complete deficiency of the enzyme (classic galactosemia) and those with partial transferase deficiency. **Classic galactosemia** is a serious disease with onset of symptoms typically by the second half of the first week of life. The incidence is approximately 1 in 60,000 live births. The newborn infant receives high amounts of lactose (up to 40% in breast milk and certain formulas), which consists of equal parts of glucose and galactose. Without the transferase enzyme, the infant is unable to metabolize galactose-1-phosphate, the accumulation of which results in injury to the liver, kidney, and brain. This injury may begin prenatally in the affected fetus by endogenous fetal production of galactose.

Clinical Manifestations

The diagnosis of uridyl transferase deficiency should be considered in newborn or young infants with any of the following features within a few days or weeks after birth: jaundice, hepatomegaly, vomiting, hypoglycemia, seizures, lethargy, irritability, feeding difficulties, poor weight gain or failure to regain birthweight, and aminoaciduria. Untreated children may show nuclear cataracts, vitreous hemorrhage, hepatic failure, cirrhosis, ascites, splenomegaly, or intellectual disability. Patients with galactosemia

are at increased risk for *Escherichia coli* neonatal sepsis; the onset of sepsis often precedes the diagnosis of galactosemia. Pseudotumor cerebri has been reported in some patients, presenting with failure to thrive and a bulging anterior fontanel. Complete withdrawal of lactose from the diet results in improvement of the acute symptoms. If untreated, death from liver failure and sepsis may follow within days. When the diagnosis is not made at birth, damage to the liver (cirrhosis) and brain (intellectual disability) becomes increasingly severe and irreversible.

Partial transferase deficiency is classified into two subtypes: clinical variant galactosemia (erythrocyte GALT enzyme activity is ≥1% of controls, but not more than 10–15%) and biochemical (Duarte) variant galactosemia (erythrocyte GALT enzyme activity is ≥25% of controls). Partial transferase deficiency is more common than classic galactosemia and is diagnosed in the newborn screening setting because of moderately elevated blood galactose and/or low transferase activity. Clinical variant galactosemia should be considered in the newborn or young infant who is not thriving or who exhibits any of the classical galactosemia-related symptoms. Generally, biochemical (Duarte) variant galactosemia is asymptomatic. There is no evidence that individuals with biochemical (Duarte) variant galactosemia have an increased risk of neurodevelopmental problems or premature ovarian insufficiency. Additional research is required to properly understand and describe the natural history of this subtype.

Diagnosis

Historically, the detection of a reducing substance in several urine specimens obtained when the patient is on a diet containing human milk, cow's milk, or any other lactose-containing formula was regarded as strongly indicative of galactosemia. The clue was detecting a reducing substance in urine by Clinitest strips (e.g., glucose, galactose) that is negative by Clinistix (which is specific for glucose only). Galactose can also be identified by chromatography or an enzymatic test specific for galactose. Galactose can be detected in urine, provided the milk feeding was within the last few hours and the child is not vomiting excessively. The addition of galactosemia to the newborn screening panel resulted in a considerable reduction in the utility of Clinitest strips for galactosemia screening. Amino acids may be detected in urine (aminoaciduria) because they are excreted together with glucose as a result of proximal renal tubular dysfunction. Because galactose is injurious to persons with galactosemia, diagnostic challenge tests dependent on administering galactose orally or intravenously should not be used. Direct enzyme assay using erythrocytes establishes the diagnosis. The clinician must confirm that the patient did not receive a blood transfusion before the collection of the blood sample for enzyme assay activity in RBCs, because this may lead to a false-negative result, and the diagnosis could be missed. The activity of galactose-1-phosphate uridyl transferase in erythrocytes can be measured in a variety of ways, one of which involves the use of a nonradioactive assay using high-performance liquid chromatography (HPLC) separation and ultraviolet (UV) light detection. Patients with glucose-6-phosphate-dehydrogenase deficiency can have false-positive results. In classic galactosemia, metabolites (urinary galactitol, red cell galactose-1-phosphate, and blood galactose concentration) may be elevated and can be used to confirm the diagnosis and monitor the response to dietary changes and metabolic control.

Genetics

GALT deficiency is an autosomal recessive disorder caused by pathogenic variants in the *GALT* gene. Based on newborn screening in the United States, the frequency of the disease is approximately 1 in 47,000 live births. There are several enzymatic variants of galactosemia. More than 327 pathogenic variants have been associated with GALT deficiency. The most common pathogenic variants that result in classic galactosemia phenotype are c.563A>G (p.Gln188Arg), c.855G>T (p.Lys285Asn), and c.584T>C (p.Leu195Pro). In Whites, the p.Gln188Arg variant is linked to an increased incidence of severe disease, premature ovarian insufficiency, and speech problems in a homozygous state. In Blacks p.Ser135Leu is common, which results in a milder phenotype (clinical variant galactosemia) despite the absence of measurable GALT activity in erythrocytes. These patients retain 10% of enzyme activity in the liver and intestinal mucosa. When treated early, Blacks who are homozygous for this variant are not at risk for neonatal *E. coli* sepsis or chronic complications (i.e., premature ovarian insufficiency and language delay). Four pathogenic variants are unique to the Duarte variant (D2): a four–base pair (bp) deletion in the GALT promoter region (c.-119-116delGTCA), a c.378-27G>C, a c.508-24G>A, and a c.507+62G>A. A fifth variant is a single amino acid substitution (c.940A>G, p.Asn314Asp, also called *N314D*), which has been observed in both the Duarte variant and functionally normal GALT alleles. Individuals heterozygous for Duarte variant galactosemia typically have 25% of normal galactose activity. Symptoms are minimal or absent, metabolites range from elevated to normal, and there is no need for intervention. Carrier testing and prenatal diagnosis can be performed by molecular testing or, less commonly, direct enzyme assay on amniocytes or chorionic villi.

Treatment and Prognosis

Consensus guidelines recommend treating patients with an RBC GALT enzyme activity of ≤10% and erythrocyte galactose-1-phosphate concentration of >10 mg/dL. The decision of whether to treat those with 10–15% RBC residual GALT activity is still a matter of debate. All galactose-containing foods should be removed from the diet on initial suspicion of galactosemia. Various non–lactose-containing milk substitutes are available (casein hydrolysates, soybean-based formula). A galactose-restricted diet, along with adequate calcium and vitamin D supplementation, reverses growth failure and hepatic dysfunction. Cataracts regress, and most patients have no persistent impairment of vision. Early diagnosis and treatment have improved the prognosis of galactosemia. On long-term follow-up, patients still manifest ovarian failure with primary or secondary amenorrhea, decreased bone mineral density, developmental delay, and learning disabilities that increase in severity with age. *Hypergonadotropic hypogonadism* is reported in 80–90+% of female patients with classic galactosemia. Although most females with classic galactosemia are infertile when they reach childbearing age, a small number have given birth. Females with the Duarte variant galactosemia and who are homozygous for p.Ser135Leu do not develop premature ovarian insufficiency. Most patients manifest speech disorders, whereas a smaller number demonstrate poor growth and impaired motor function and balance (with or without overt ataxia). Strict dietary restriction and relative control of galactose-1-phosphate levels do not always correlate with long-term outcome, leading to the belief that other factors such as elevated galactitol, decreased uridine diphosphate galactose (a donor for galactolipids and proteins), and endogenous galactose production may be responsible.

GALACTOKINASE DEFICIENCY

The deficient enzyme is galactokinase, which normally catalyzes the phosphorylation of galactose. The principal metabolites accumulated are galactose and galactitol. Two genes encode enzymes with galactokinase activity, *GALK1* and *GALK2,* although pathogenic variants have been noted only in *GALK1* that cause autosomal recessive galactokinase deficiency. **Cataract** is the most common manifestation of galactokinase deficiency, and pseudotumor cerebri is a rare complication. The incidence of hypoglycemia and infection is comparable to those found in the general population. There is a higher rate of bleeding diathesis, encephalopathy, and elevated liver transaminases than in the general population during the newborn period. Some patients have intellectual impairment and motor and language delays. Heterozygous carriers may be at risk for presenile cataracts. Laboratory findings include increased concentration of blood galactose levels and urinary galactitol, provided the infant has been fed a lactose-containing formula. The diagnosis is made by demonstrating an absence of galactokinase activity in erythrocytes or fibroblasts. GALT activity is normal. Treatment is dietary restriction of galactose.

URIDINE DIPHOSPHATE GALACTOSE-4-EPIMERASE DEFICIENCY

There are three distinct forms of **epimerase** deficiency based on the level of enzyme activity in different cell types. The first is a **benign** form known as *peripheral epimerase deficiency galactosemia,* diagnosed incidentally through newborn screening programs. Affected individuals are asymptomatic because the enzyme deficiency is limited to

leukocytes and erythrocytes. This form does not require treatment. The second subtype is called **intermediate** form and is caused by epimerase deficiency in RBCs and circulating white blood cells, as well as epimerase activity below 50% of normal in all other cells. Neonates with the intermediate form are typically asymptomatic, even when fed a normal milk diet, and are diagnosed only as a result of newborn screening. The long-term consequences remain unknown. During infancy and early childhood, these individuals are treated with a galactose-/lactose-restricted diet. The third form is rarer and more **severe** as a result of generalized epimerase deficiency. Clinical manifestations resemble GALT transferase deficiency, with additional symptoms of hypotonia and nerve deafness. Clinical symptoms improve with restriction of galactose/lactose in the diet. Although this severe form of epimerase galactosemia is rare, it must be considered in a symptomatic patient with elevated RBC galactose-1-phosphate, urinary galactose, and galactitol levels but with normal GALT transferase activity. The abnormally accumulated metabolites are similar to those in transferase deficiency, with the addition of an increase in cellular uridine diphosphate (UDP) galactose. Biochemical diagnosis is confirmed by the assay of epimerase in erythrocytes demonstrating reduced activity. UDP galactose-4-epimerase is encoded by the *GALE* gene, and pathogenic biallelic variants result in autosomal recessive disease. Carrier detection is possible by measurement of epimerase activity in the erythrocytes. Prenatal diagnosis for the severe form of epimerase deficiency can be done using an enzyme assay of cultured amniotic fluid cells or testing of known familial pathogenic variants.

Patients with the severe form of epimerase deficiency cannot synthesize UDP galactose from UDP glucose and are galactose dependent. Because galactose is an essential component of many nervous system structural proteins, patients are placed on a galactose-*restricted* diet rather than a galactose-*free* diet. Galactose restriction is also indicated for infants and young children with the intermediate form. Additional outcome data are needed to optimize these approaches and develop a better understanding of long-term issues in this patient population. Infants with mild epimerase deficiency have not required treatment.

Galactose Mutarotase Deficiency

GALM catalyzes the first step in the Leloir pathway, the conversion of beta-D-galactose to alpha-D-galactose. There have been a few cases of GALM deficiency reported, with an overall estimated incidence of GALM deficiency in all populations of less than 1 in 200,000, with higher incidences in Black and Japanese populations. Apart from cataracts, individuals with GALM deficiency are often healthy. In some reported cases, elevated blood galactose-1-phosphate (Gal-1-P) and galactose levels were identified during newborn screening. There are no reports of long-term implications of this condition and no formal recommendations regarding optimal dietary intake in this population.

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107.3 Defects in Fructose Metabolism

Ghada Hijazi and Priya S. Kishnani

Two inborn errors are known in the specialized pathway of fructose metabolism: benign or essential fructosuria and hereditary fructose intolerance. Fructose-1,6-bisphosphatase deficiency, not formally a defect of the specialized fructose pathway, is discussed in Chapter 107.4.

DEFICIENCY OF FRUCTOKINASE (ESSENTIAL OR BENIGN FRUCTOSURIA)

Deficiency of fructokinase is not associated with clinical manifestations. **Fructosuria** is an incidental finding identified in asymptomatic individuals with reducing substances in urine. No treatment is necessary, and the prognosis is excellent. Inheritance of this autosomal recessive trait is caused by biallelic variants in the *KHK* gene, with an incidence of 1 in 120,000 live births.

Fructokinase catalyzes the first step of metabolism of dietary fructose—conversion of fructose to fructose-1-phosphate (see Fig. 107.1). Without this enzyme, fructose consumption results in a rise in the blood level of fructose, which is partially excreted in the urine, as there is little to no renal threshold for fructose. In adipose tissue and muscle, hexokinase converts the remainder to fructose-6-phosphate, which is considered a slower alternative process. Clinitest results reveal the urinary reducing substance, which can be identified as fructose by chromatography.

DEFICIENCY OF FRUCTOSE-1-PHOSPHATE ALDOLASE (ALDOLASE B, HEREDITARY FRUCTOSE INTOLERANCE)

In comparison to fructokinase, deficiency of fructose-1-phosphate aldolase **(aldolase-B)** is a severe condition in infants resulting from a deficiency of activity in the liver, kidney, and intestine. Fructose-1-phosphate aldolase hydrolyzes fructose-1-phosphate to glyceraldehyde and dihydroxyacetone phosphate. In the glycolytic-gluconeogenic pathway, this enzyme catalyzes the hydrolysis of fructose-1,6-bisphosphate to dihydroxyacetone phosphate and glyceraldehyde phosphate. In the absence of aldolase B activity, there is a rapid accumulation of fructose-1-phosphate and depletion of ATP, which presents with severe symptoms when fructose-containing food is ingested. Accumulation of fructose-1-phosphate also results in inhibition of gluconeogenesis (via inhibition of aldolase A) and glycogenolysis (via inhibition of glycogen phosphorylase A), resulting in decreased glucose production and a rapid decline in blood glucose. Because isozyme fructose-1,6-bisphosphate aldolase (aldolase A) metabolizes fructose-1,6-bisphosphate, glycolysis and gluconeogenesis are not affected in patients with hereditary fructose intolerance in the fasting state.

Epidemiology and Genetics

The incidence of **hereditary fructose intolerance (HFI)** is estimated to be as high as 1 in 26,000 live births. HFI is inherited in an autosomal recessive manner because of biallelic pathogenic variants in the *ALDOB* gene. At least 60 pathogenic variants causing HFI are known. The most common pathogenic variant identified in the Northern European population is p.Ala150Pro. This variant, along with two other missense variants (p.Ala175Asp and p.Asn335Lys), account for 80–85% of HFI cases in Europe and the United States. The diagnosis of HFI can be made by gene sequencing or demonstration of hepatic fructose-1-phosphate aldolase (aldolase B) activity deficiency on liver biopsy (uncommon).

Clinical Manifestations

Affected individuals remain asymptomatic until fructose, sucrose (table sugar), or sorbitol is introduced in the diet (usually from fruit, fruit juice, or sweetened cereal). Signs and symptoms typically manifest in infancy when foods or formulas containing these sugars are introduced. Certain patients are very sensitive to fructose, whereas others can tolerate moderate intake (up to 250 mg/kg/day). Early clinical manifestations resemble galactosemia and include jaundice, hepatomegaly, vomiting, lethargy, irritability, and convulsions. There may also be a higher incidence of celiac disease in patients with HFI (>10%) compared with the general population (1–3%). With age, affected individuals typically develop an aversion to fructose-containing foods because of associated symptoms of nausea, vomiting, and abdominal pain.

Characteristic laboratory findings include lactic acidosis, hypophosphatemia, hyperuricemia, and hypermagnesemia. A prolonged prothrombin time, hypoalbuminemia, elevation of bilirubin and transaminase levels, and proximal tubular dysfunction are also seen. Acute fructose ingestion produces symptomatic hypoglycemia as a result of impaired gluconeogenesis, with higher intakes causing a more severe clinical picture. Chronic ingestion results in failure to thrive and hepatic disease. If the intake of fructose persists, hypoglycemic episodes recur, leading to progressive renal and hepatic failure and eventually death.

Diagnosis

The presence of a reducing substance in urine during an acute episode raises the possibility of HFI. Oral fructose challenge is no longer considered a diagnostic approach because of the high risk to the patient,

who can become acutely ill after the test. A definitive diagnosis is made by demonstration of two pathogenic variants in *ALDOB* on molecular genetic testing. A common pathogenic variant (p.Ala150Pro) accounts for 53% of HFI alleles worldwide. An alternative approach to diagnosis is to demonstrate deficient hepatic fructose-1-phosphate aldolase (aldolase B) activity on liver biopsy. Carbohydrate-deficient transferrin (CDT) testing (see Chapter 107.7) is generally abnormal in patients with HFI and can be used to monitor fructose, sucrose, and sorbitol consumption in the diet.

Treatment

Acute episodes are managed symptomatically by correcting hypoglycemia with IV glucose (dextrose) administration, providing supportive treatment of hepatic and/or renal insufficiency, and correcting metabolic acidosis. Complete elimination of fructose usually rapidly reverses symptoms and results in normalization of related metabolic disturbances. The cornerstone of long-term treatment is the complete restriction of all sources of fructose, sucrose, and sorbitol from the diet. It may be difficult because these sugars are widely used additives, found even in many medicinal preparations. With treatment, liver and kidney dysfunction improve, and catch-up in growth is common. Intellectual development is usually unimpaired. As the patient matures, symptoms become milder even after fructose ingestion, and the long-term prognosis is good. Because of voluntary dietary avoidance of sucrose, affected patients have few dental caries. Care should be taken to avoid fructose-containing IV fluids during hospitalizations. Regular supplementation with a "sugar-free" multivitamin is required to avoid micronutrient deficiencies resulting from reduced fruit and vegetable consumption.

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107.4 Defects in Intermediary Carbohydrate Metabolism Associated with Lactic Acidosis

Ghada Hijazi and Priya S. Kishnani

Lactic acidosis **(type B3)** occurs with inborn errors of metabolism, including defects of carbohydrate metabolism, that interfere with the conversion of pyruvate to glucose via the pathway of *gluconeogenesis* or to carbon dioxide and water via the mitochondrial enzymes of the Krebs cycle. Figure 107.4 depicts the relevant metabolic pathways. Type I GSD, fructose-1,6-bis phosphatase deficiency, and phosphoenolpyruvate carboxylase deficiency are disorders of gluconeogenesis associated with lactic acidosis. Other metabolic disorders, including mitochondrial respiratory chain defects, pyruvate dehydrogenase complex deficiency, pyruvate carboxylase deficiency, defects of fatty acid oxidation, organic acidurias (see Chapters 105.6, 105.10, and 106.1), biotin synthesis, and utilization defects also can cause lactic acidosis (Table 107.2). Some of these disorders are easily distinguishable by the presence of an abnormal acylcarnitine profile, amino acids in the blood, and urine organic acids. Blood lactate, pyruvate, acylcarnitine profile, and urine organic acids should be ordered in infants and children with unexplained acidosis, especially if there is an increased anion gap.

Lactic acidosis unrelated to an enzymatic defect occurs in conditions associated with hypoxemia and/or hypoperfusion (**type A** lactic acidosis). In this case, and in defects in the respiratory chain, the serum pyruvate concentration may remain normal (<1.0 mg/dL, with increased lactate to pyruvate ratio), whereas pyruvate is usually increased when lactic acidosis results from an enzymatic defect in gluconeogenesis or pyruvate dehydrogenase complex (both lactate and pyruvate are increased, and the ratio is normal). Lactate and pyruvate should be measured in the same blood specimen and on multiple blood specimens obtained when the patient is symptomatic because lactic acidosis can be intermittent. Figure 107.5 is an algorithm for the differential diagnosis of lactic acidosis. Lactic acidosis is also noted with various underlying diseases **(type B1)** and drugs or toxins **(type B2)** (see Table 107.2).

DISORDERS OF GLUCONEOGENESIS

Deficiency of Glucose-6-Phosphatase (Type I Glycogen Storage Disease)

Type I GSD is associated with significant fasting lactic acidosis. The chronic metabolic acidosis predisposes these patients to osteopenia; acute lactic acidosis associated with hypoglycemia is a life-threatening condition in GSD I (see Chapter 107.1).

Fructose-1,6-Bisphosphatase Deficiency

Fructose-1,6-bisphosphatase (FBP) deficiency impairs the formation of glucose from all gluconeogenic precursors, including dietary fructose. Hypoglycemia occurs when glycogen reserves are limited or exhausted. The **clinical manifestations** are characterized by life-threatening episodes of acidosis, hypoglycemia, hyperventilation,

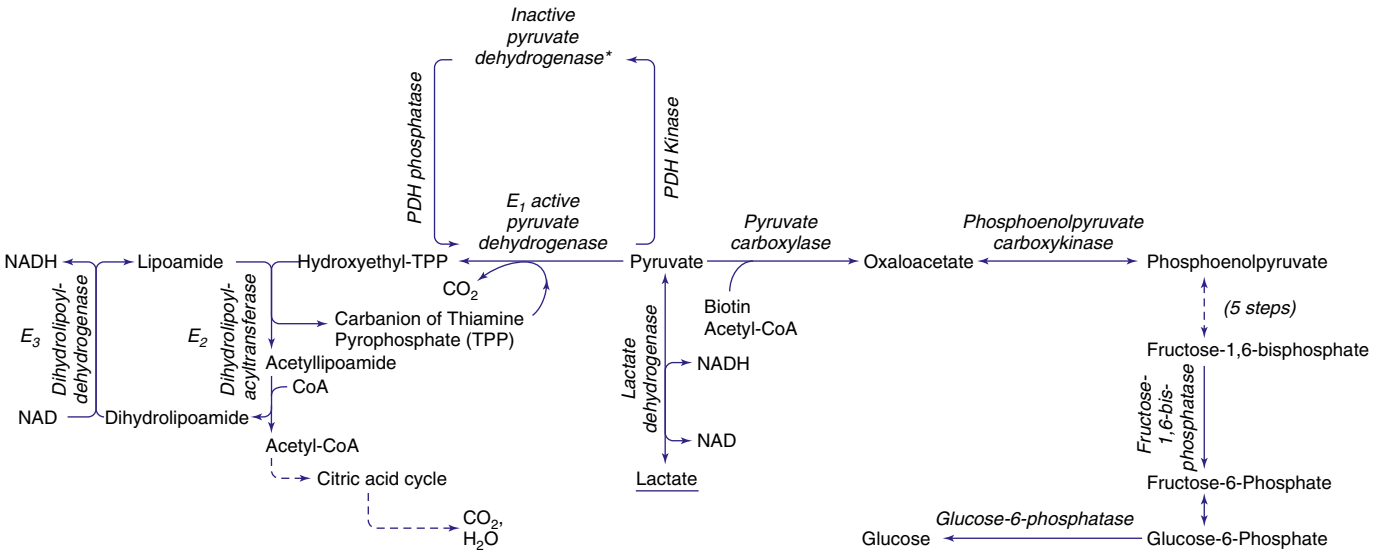

Fig. 107.4 Enzymatic reactions of carbohydrate metabolism, deficiencies of which can give rise to lactic acidosis, pyruvate elevations, or hypoglycemia. The pyruvate dehydrogenase (PDH) complex comprises, in addition to E_1, E_2, and E_3, an E_3-binding protein (not shown), previously called protein X, and PDH kinase and phosphatase. NAD/NADH, nicotinamide adenine dinucleotide.

Table 107.2 Causes of Type B Lactic Acidosis

TYPE B1—UNDERLYING DISEASES
Renal failure
Hepatic failure
Diabetes mellitus
Malignancy
Systemic inflammatory response syndrome
Human immunodeficiency virus

TYPE B2—DRUGS AND TOXINS
Acetaminophen
Alcohols: ethanol, methanol, diethylene glycol, isopropanol, and propylene glycol
Antiretroviral nucleoside analogs—zidovudine, didanosine, and lamivudine
β-Adrenergic agonists: epinephrine, ritodrine, and terbutaline
Biguanides: phenformin and metformin
Cocaine, methamphetamine
Cyanogenic compounds: cyanide, aliphatic nitriles, and nitroprusside
Diethyl ether
Fluorouracil
Halothane
Iron
Isoniazid
Linezolid
Nalidixic acid
Niacin
Propofol
Salicylates
Strychnine
Sugars and sugar alcohols: fructose, sorbitol, and xylitol
Sulfasalazine
Total parenteral nutrition
Valproic acid
Vitamin deficiencies: thiamine and biotin

TYPE B3—INBORN ERRORS OF METABOLISM
Glucose-6-phosphatase deficiency (von Gierke disease)
Fructose-1,6-diphosphatase deficiency
Phosphoenolpyruvate carboxykinase deficiency
Pyruvate carboxylase deficiency
Pyruvate dehydrogenase complex (PDHC) deficiency
Krebs cycle defects
Methylmalonic aciduria and other organic acidemias
Kearns-Sayre syndrome
Pearson syndrome
Barth syndrome
Mitochondrial DNA depletion syndromes
Nuclear DNA respiratory chain defects
Mitochondrial DNA respiratory defects
Mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes (MELAS)
Myoclonic epilepsy with ragged red fibers (MERRF)

Adapted from Vernon C, LeTourneau JL. Lactic acidosis: recognition, kinetics, and associated prognosis. *Crit Care Clin.* 2010;26:255–283, Box 1.

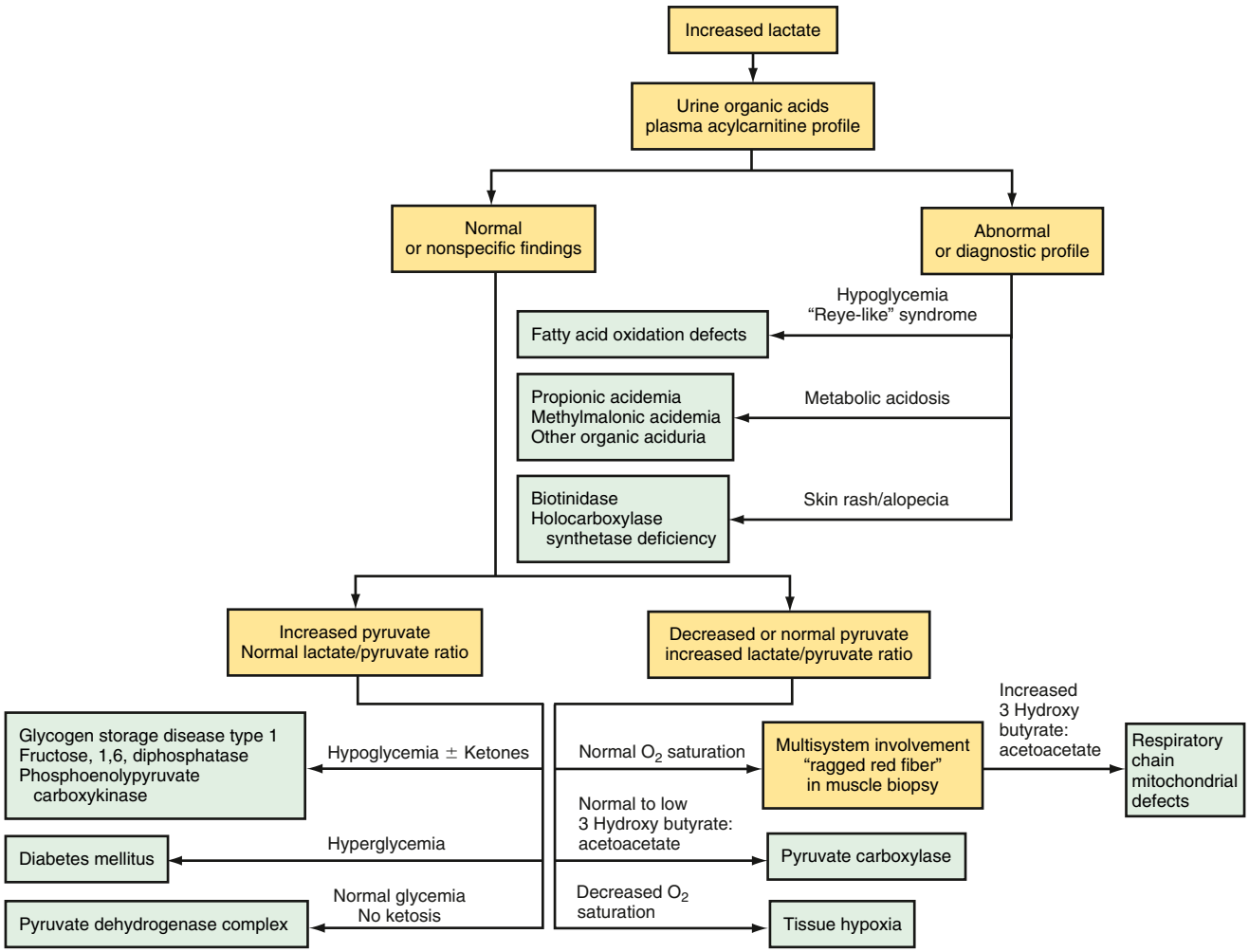

Fig. 107.5 Algorithm of the differential diagnosis of lactic acidosis.

seizures, and coma. In about half of the cases, the deficiency presents in the first week of life. In certain cases, variable degrees of liver impairment (elevated liver tests) and/or failure have been documented, requiring supportive therapy, including intravenous glucose infusion. In infants and small children, hypoglycemia and/or hepatopathy-like episodes are triggered by fasting or missing meals, febrile infections, gastroenteritis, vomiting, and/or poor oral intake. The frequency of these episodes decreases with age. Laboratory findings include low blood glucose, high lactate, glycerol, and uric acid levels and high anion gap metabolic acidosis. Because of the inability of current biochemical assays for plasma triglycerides to differentiate glycerol from triglycerides, FBP deficiency is usually associated with elevated triglyceride levels. This phenomenon is referred to as *pseudo-hypertriglyceridemia* because the plasma has a high concentration of glycerol rather than triglycerides. In contrast to HFI, there is usually no oral aversion to sweets and fruit. Between episodes, renal tubular and liver function may be normal; however, long-term studies are limited.

The **diagnosis** is established by noninvasive molecular genetic testing for pathogenic variants or demonstrating an enzyme deficiency in either liver biopsy or mononuclear white blood cells. The gene coding for fructose-1,6-bisphosphatase is *FBP1,* and pathogenic variants are known, making carrier detection and prenatal diagnosis possible. **Treatment** of acute attacks consists of correction of hypoglycemia and acidosis by IV glucose infusion, and the response is usually rapid. Avoidance of fasting, frequent feedings, aggressive management of infections, and restriction of fructose, sucrose, glycerol, and/or sorbitol from the diet can prevent further episodes. For long-term prevention of hypoglycemia, a slowly released carbohydrate such as cornstarch is useful. Children remain asymptomatic in between crises, and the majority have normal growth and psychomotor development. A few children have been documented to have suffered brain injury and/or cognitive disabilities, most likely as a result of early and untreated hypoglycemia.

Phosphoenolpyruvate Carboxykinase Deficiency

Phosphoenolpyruvate carboxykinase **(PEPCK)** is a key enzyme in gluconeogenesis. It catalyzes the conversion of oxaloacetate to phosphoenolpyruvate and carbon dioxide (see Fig. 107.4). PEPCK deficiency may occur as a cytosolic or mitochondrial enzyme deficiency, as it is encoded by two distinct genes (*PCK1* and *PCK2,* respectively).

PEPCK deficiency has been reported in only a few cases. The **clinical features** are heterogeneous, with hypoglycemia, lactic acidemia, hepatomegaly, hypotonia, developmental delay, and failure to thrive as the major manifestations. There may be multisystem involvement, with neuromuscular deficits, hepatocellular damage, renal dysfunction, and cardiomyopathy. The **diagnosis** is based on the reduced activity of PEPCK in the liver, fibroblasts, or lymphocytes. Fibroblasts and lymphocytes are not suitable for diagnosing the cytosolic form of PEPCK deficiency because these tissues possess only mitochondrial PEPCK. PEPCK deficiency can also occur as a secondary phenomenon caused by other disease, such as mitochondrial depletion disorders. As a result, molecular testing confirming the presence of two pathogenic variants in either the *PCK1* or *PCK2* gene is the recommended gold standard for diagnosis. To avoid hypoglycemia, patients should receive **treatment** with slow-release carbohydrates such as cornstarch, and fasting should be avoided.

DISORDERS OF PYRUVATE METABOLISM

Pyruvate is formed from glucose and other monosaccharides, from lactate, and from alanine. It is metabolized through four main enzyme systems: LDH, ALT, pyruvate carboxylase, and pyruvate dehydrogenase complex. Deficiency of the M subunit of LDH causes exercise intolerance and myoglobinuria (see Chapter 107.1).

Pyruvate Dehydrogenase Complex Deficiency

After entering the mitochondria, pyruvate is converted into acetyl-CoA by the pyruvate dehydrogenase complex **(PDHC)** and then enters the tricarboxylic acid cycle for ATP production. The complex comprises three functional enzymes: $\mathbf{E_1}$, an α-ketoacid decarboxylase; $\mathbf{E_2}$, a dihydrolipoyl acyltransferase; and $\mathbf{E_3}$, a dihydrolipoyl dehydrogenase. The complex also contains an E_3-binding protein (previously called **protein X**) and PDHC kinase and phosphatase, which regulate the complex's activity via phosphorylation/dephosphorylation. Additionally, it requires thiamine pyrophosphate (TPP), lipoic acid, and flavin adenine dinucleotide (FAD) as cofactors. The E_1 subunit, which is made up of two subunits, $E_{1\alpha}$ and $E_{1\beta}$, catalyzes the first and rate-limiting step in the reaction. The most common disorder is caused by a gene defect in the $E_{1\alpha}$ subunit (see Fig. 107.4).

Deficiency of the PDHC is the most common of the disorders leading to lactic acidemia and CNS anomalies and dysfunction. The CNS dysfunction occurs because the brain obtains its energy primarily from oxidation of glucose through glycolysis. Brain acetyl-CoA is synthesized almost exclusively from pyruvate.

PDHC deficiency is mostly caused by pathogenic variants in the gene coding for the $E_{1\alpha}$ subunit, which is inherited in an X-linked dominant fashion; therefore both males and females with a pathogenic variant exhibit features.

Clinical Manifestations

PDHC deficiency has a wide spectrum of presentations, from the most severe neonatal presentation to a mild late-onset form. The **neonatal onset** is associated with lethal lactic acidosis, white matter cystic lesions, agenesis of the corpus callosum, and severe enzyme deficiency. **Infantile/childhood onset** can be lethal or associated with psychomotor delay and chronic lactic acidosis, cystic lesions in the brainstem and basal ganglia, and features of **Leigh syndrome** (see later and Chapter 638.2). **Late onset** cases, including males, may have less acidosis, greater enzyme activity, and episodic ataxia upon consumption of high-carbohydrate-containing foods. Intelligence in affected males may be normal. Developmental delay, hypotonia, hypertonia, seizures, ataxia, peripheral neuropathy, dystonia, dyskinesia, and/or hemiplegia are among neurologic symptoms of PDHC deficiency. Patients of all ages may have dysmorphic facial features, resembling those seen in fetal alcohol syndrome, as a result of PDHC inhibition and reduction of energy metabolism in the latter. Typically, patients with $\mathbf{E_{1\beta}}$ deficiency are more severely affected.

The $\mathbf{E_2}$ deficiency is rare and results in a milder phenotype with varying brain MRI findings and survival into adolescence and adulthood.

The $\mathbf{E_3}$ **lipoamide dehydrogenase** deficiency leads to reduced activity not only in the PDHC but also in the α-ketoglutarate and branched-chain ketoacid dehydrogenase complexes. This deficiency is more common in the Ashkenazi Jewish population. Individuals deficient in E_3 may present with hypotonia and lactic acidosis during infancy, and affected infants typically die during the first few years of life. Surviving children have been shown to have growth delay and severe neurologic problems. Hepatic disease could be a component of this phenotype or may manifest separately. Only a small number of patients exhibit a myopathic phenotype including muscle weakness and elevated CK. In urine organic acid analysis, α-ketoglutarate and branched-chain ketoacids can be detected, and plasma amino acid analysis may reveal elevated levels of leucine, isoleucine, valine, and allo-isoleucine. These findings depend greatly on the phenotype of the disease. The $\mathbf{E_3}$ **binding protein deficiency** can present with either neonatal lactic acidosis, severe psychomotor retardation, or seizure, as well as abnormalities on brain MRI. **Pyruvate dehydrogenase phosphatase** and kinase deficiencies have also been reported. These other PDHC defects have clinical manifestations within the variable spectrum associated with PDHC deficiency caused by $E_{1\alpha}$ deficiency.

Treatment

The general prognosis is poor, except in rare patients whose variants are associated with altered affinity for thiamine pyrophosphate because they may respond to thiamine supplementation. Because carbohydrates can aggravate lactic acidosis, a ketogenic diet is recommended. The ketogenic diet has been found to decrease blood lactate levels and to reduce seizures and improve ataxia and sleep habits. Modified ketogenic diets (a 1:1 fat-to-carbohydrate and protein ratio) have also been

helpful in patients who cannot tolerate a strict ketogenic diet. Infusions of glucose, lactated Ringer, or other drugs known to increase the risk of lactic acidosis, such as metformin, are contraindicated. A potential treatment strategy is to maintain any residual PDHC in its active form by oral administration of dichloroacetate, an inhibitor of E_1 kinase. Beneficial effects of controlling postprandial lactic acidosis have been shown in some patients. Young children generally tolerate **dichloroacetate** well, but continued exposure is associated with peripheral neuropathy, a condition that could be attributable to the drug or the disease. In vitro studies have demonstrated that **phenylbutyrate** increases PDC enzyme activity, especially in those with pathogenic variants linked to residual enzyme activity. In patients with E_3 deficiency, **branched-chain amino acid** restriction and **riboflavin** supplementation are recommended.

Deficiency of Pyruvate Carboxylase

Pyruvate carboxylase is a mitochondrial, biotin-containing enzyme essential in the process of gluconeogenesis; it catalyzes the conversion of pyruvate to oxaloacetate. The enzyme is also essential for Krebs cycle function as a provider of oxaloacetate and is involved in lipogenesis and formation of nonessential amino acids. **Clinical manifestations** of this deficiency have varied from neonatal severe lactic acidosis accompanied by hyperammonemia, hypercitrullinemia, hyperlysinemia, and hypoglycemia **(type B)** to late-onset mild to moderate lactic acidosis and developmental delay **(type A)**. In both types, patients who survive usually have severe psychomotor retardation with seizures, spasticity, and microcephaly. Some patients have pathologic changes in the brainstem and basal ganglia that resemble **Leigh syndrome**. The clinical severity appears to correlate with the level of the residual enzyme activity. A "benign" form of pyruvate carboxylase deficiency has also been described, characterized by recurrent attacks of lactic acidosis and ketoacidosis, as well as mild neurologic deficits **(type C)**. Laboratory findings include elevated levels of blood lactate, pyruvate, and alanine and ketonuria. In type B, the lactate-to-pyruvate ratio is increased; this ratio is normal in types A and C. Additionally, in type B, blood ammonia, citrulline, and lysine levels are elevated with low glutamine, which might suggest a primary anaplerotic defect involving the urea cycle. The mechanism is likely the result of depletion of oxaloacetate, which leads to reduced levels of aspartate, a substrate for argininosuccinate synthase in the urea cycle (see Chapter 105.12). The gene for pyruvate carboxylase is *PC,* and many pathogenic variants have been identified.

Diagnosis of pyruvate carboxylase deficiency is made by the measurement of enzyme activity in cultured skin fibroblasts or lymphoblasts and must be differentiated from holocarboxylase synthase (HCS) or biotinidase deficiency. Molecular genetic testing is presently the preferred approach for diagnosis. **Treatment** consists of avoidance of fasting and modifying the diet to include high amounts of carbohydrate and protein. During acute episodes of lactic acidosis, patients should receive continuous IV glucose. Metabolic abnormalities have been reduced with the use of anaplerotic compounds such as citrate, aspartate, or more recently, triheptanoin, but no improvement has been shown in neurologic symptoms or increased life span. Although liver transplantation has been shown to correct biochemical abnormalities in two patients, the long-term benefits of this procedure are still unknown. Biotin is a cofactor used for this condition, but most patients do not respond to biotin. Lactated Ringer solution is contraindicated in this condition because of the theoretical risk of worsening lactic acidosis in these patients.

Deficiency of Pyruvate Carboxylase Secondary to Deficiency of Holocarboxylase Synthase or Biotinidase

Deficiency of either **HCS** or biotinidase, enzymes involved in biotin metabolism and recycling, respectively, results in multiple-carboxylase deficiency (pyruvate carboxylase, acetyl-CoA carboxylase, 3-methyl-crotonyl-CoA carboxylase, and propionyl-CoA carboxylase) and in **clinical manifestations** associated with the respective deficiencies, including rash, lactic acidosis, and alopecia (see Chapter 103.6). Both enzyme deficiencies are autosomal recessive disorders. The incidence of HCS and biotinidase deficiency is approximately 1 in 87,000 and 1 in 80,000 live births, respectively. Ancestry-specific pathogenic variants in the HCS and biotinidase (BTD) genes have been described. Two common pathogenic variants (c.98_104delinsTCC; p.Cys33PhefsTer36 and c.1612C>T; p.Arg538Cys) in the BTD account for 52% of all pathogenic alleles in symptomatic patients with biotinidase deficiency.

The course of HCS may be more protracted and less responsive to therapy than BTD deficiency. Lack of treatment may be associated with intermittent exacerbations on top of chronic lactic acidosis, failure to thrive, seizures, and hypotonia leading to spasticity, lethargy, coma, and death. Auditory and optic nerve dysfunction can lead to deafness and blindness, respectively. The course of BTD deficiency may be severe if left untreated. Late-onset milder forms have also been reported. Laboratory findings include an anion gap metabolic acidosis, lactic acidosis, and abnormal urine organic acids. Lactate, 3-methylcrotonylglycine, 3-hydroxypropionate, 3-hydroxisovalerate, and methyl citrate may accumulate in urine organic acid chromatography (OAC), though the concentrations of metabolites differ according to the severity of the enzyme deficiency. Blood and/or urine biotin levels can be used to evaluate treatment adherence, but they do not contribute to diagnosis. Biotin concentrations in plasma and urine are normal in patients with HCS deficiency and are often decreased in patients with BTD deficiency, particularly when the assay used does not detect biocytin.

Diagnosis of HCS deficiency and biotinidase deficiency can be detected through newborn screening tandem mass spectroscopy, which enables early treatment and improves outcomes. Molecular genetic testing is required to confirm the diagnosis, which involves the detection of two pathogenic variants. BTD deficiency can be diagnosed by reduced enzyme activity in the patient's serum or plasma, which helps to classify the deficiency into two categories: profound deficiency (biotinidase activity less than 10% of mean normal serum BTD activity) and partial deficiency (BTD activity between 10% and 30% of mean normal serum BTD activity). HCS activity in skin fibroblasts or lymphocytes can be assessed when HCS deficiency is suspected but genetic testing is equivocal.

Treatment

Treatment consists of biotin supplementation 5-20 mg/day and is generally effective if started early before the onset of symptoms or development of brain damage. Newborn screening has facilitated early treatment for patients with partial or profound BTD deficiency, who respond well to 5-10 mg of biotin; some patients may require higher doses during acute illness. Patients identified through newborn screening and treated with biotin have remained asymptomatic.

Mitochondrial Respiratory Chain Defects (Oxidative Phosphorylation Disease)

The mitochondrial respiratory chain catalyzes the oxidation of fuel molecules and transfers the electrons to molecular oxygen, with concomitant energy transduction into ATP **(oxidative phosphorylation)** (see Chapter 108). The respiratory chain produces ATP from adenosine diphosphate and inorganic phosphate, using the energy from electrons transferred from nicotinamide adenine dinucleotide (NADH) or flavin adenine dinucleotide, and includes five specific complexes (I: NADH–coenzyme Q reductase; II: succinate–coenzyme Q reductase; III: coenzyme QH2 cytochrome-c reductase; IV: cytochrome-c oxidase; V: ATP synthase). Each complex is composed of 4-35 individual proteins that are encoded by nuclear or maternally inherited mitochondrial DNA, with the exception of complex II, which is encoded solely by nuclear genes. Alterations of these complexes or assembly systems produce chronic lactic acidosis, presumably because of a change in the reduction-oxidation state with increased concentrations of NADH (Table 107.3).

In contrast to PDHC or pyruvate carboxylase deficiency, skeletal muscle and the heart are usually involved in the respiratory chain disorders. On muscle biopsy, **ragged red fibers** indicating mitochondrial proliferation are suggestive of mitochondrial involvement when present, especially when observed in young patients with symptoms (see

Table 107.3 Clinical and Genetic Heterogeneity of Disorders Related to Pathogenic Variants in Mitochondrial DNA*

SYMPTOMS, SIGNS, AND FINDINGS	LARGE DELETIONS IN MITOCHONDRIAL DNA			PATHOGENIC VARIANT IN TRANSFER RNA		PATHOGENIC VARIANT IN RIBOSOMAL RNA	PATHOGENIC VARIANT IN MESSENGER RNA		
	KSS	PEO	PS	MERRF	MELAS	AID	NARP	MILS	LHON
CENTRAL NERVOUS SYSTEM									
Seizures	−	−	±	+++	+	−	±	+	−
Ataxia	+	−	±	+	+		+	±	−
Myoclonus	−	−	−	+	±	−	−	−	−
Psychomotor retardation	−	−	±	−	±	−	−	+	−
Psychomotor regression	+	−	−	±	+	−	±	+	−
Hemiparesis and hemianopia	−	−	−	−	+++	−	−	−	−
Cortical blindness	−	−	−	−	+	−	−	−	−
Migraine-like headaches	−	−	−	±	+	−	−	−	−
Dystonia	−	−	−	−	+	−	−	+	±
PERIPHERAL NERVOUS SYSTEM									
Peripheral neuropathy	±	−	−	±	±	−	+	±	±
MUSCLE									
Weakness ± exercise intolerance	+	+++	±	+	+	−	+	+	±
Ophthalmoplegia	+	+	±	±	±	−	±	±	−
Ptosis	+	+	±	±	−	−	−	±	−
EYE									
Pigmentary retinopathy	+	−	−	±	±	−	+	±	−
Optic atrophy	−	−	−	±	±	−	±	±	+
BLOOD									
Sideroblastic anemia	-	−	+	−	−	−	−	−	−
ENDOCRINE SYSTEM									
Diabetes mellitus	±	−	±	±	±	−	−	±	−
Short stature	+	−	±	+	+	−	±	−	−
Hypoparathyroidism	±	−	±	−	±	−	−	−	−
HEART									
Conduction disorder	+	−	−	±	±	−	±	±	±
Cardiomyopathy	±	−	−	±	±	+	−	±	−
GASTROINTESTINAL SYSTEM									
Exocrine pancreatic dysfunction	±	−	±	−	−	−	−	−	−
Intestinal pseudoobstruction	−	−	−	−	±	−	−	±	−
EAR, NOSE, AND THROAT									
Sensorineural hearing loss	±	−	−	+	+	+	±	±	−
KIDNEY									
Fanconi syndrome/RTA	±	−	±	−	±	−	−	±	−
LABORATORY FINDINGS									
Lactic acidosis	+	±	+	+	+	±	±	±	−
Ragged-red fibers on muscle biopsy	+	+	±	+	+	−	−	±	−
MODE OF INHERITANCE									
Maternal	−	−	−	+	+	+	+	+	+
Sporadic	+	+	+	−	−	−	−	−	−

*Characteristic constellations of symptoms and signs are in **bold**.

+, Presence of a symptom, sign, or finding; −, absence of a symptom, sign, or finding; ±, possible presence of a symptom, sign, or finding; AID, aminoglycoside-induced deafness; KSS, Kearns-Sayre syndrome; LHON, Leber hereditary optic neuropathy; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes; MERRF, myoclonic epilepsy with ragged red fibers; MILS, maternally inherited Leigh syndrome; NARP, neuropathy, ataxia, and retinitis pigmentosa; PEO, progressive external ophthalmoplegia; PS, Pearson syndrome, RTA, renal tubular acidosis.

Modified from DiMauro S, Schon EA. Mitochondrial respiratory-chain diseases. *N Engl J Med.* 2003;348:2656–2668.

Fig. 107.5). Because of the ubiquitous nature of oxidative phosphorylation, a defect of the mitochondrial respiratory chain accounts for a vast array of clinical manifestations and should be considered in patients of all ages presenting with multisystem involvement. Some deficiencies resemble **Leigh syndrome** with lactic acidosis, whereas others present with strokelike episodes, myopathies, or ataxia including **MELAS** (mitochondrial encephalopathy, lactic acidosis, and strokelike episodes), **MERRF** (myoclonic epilepsy and ragged red fibers), and **Kearns-Sayre syndrome** (external ophthalmoplegia, ptosis, metabolic acidosis, retinal degeneration, heart block, myopathy, and high cerebrospinal fluid protein) (see Table 107.3) (see Chapters 638.2 and 651.4). There is a higher incidence of neuropsychiatric disorders in adults with a primary oxidative phosphorylation disease than in the general population.

Diagnosis requires identification of pathogenic variants in mitochondrial DNA or nuclear genes encoding mitochondrial proteins or demonstrating functional abnormalities of oxidative phosphorylation enzyme complex activities in tissues (e.g., skin, muscle, or liver). In

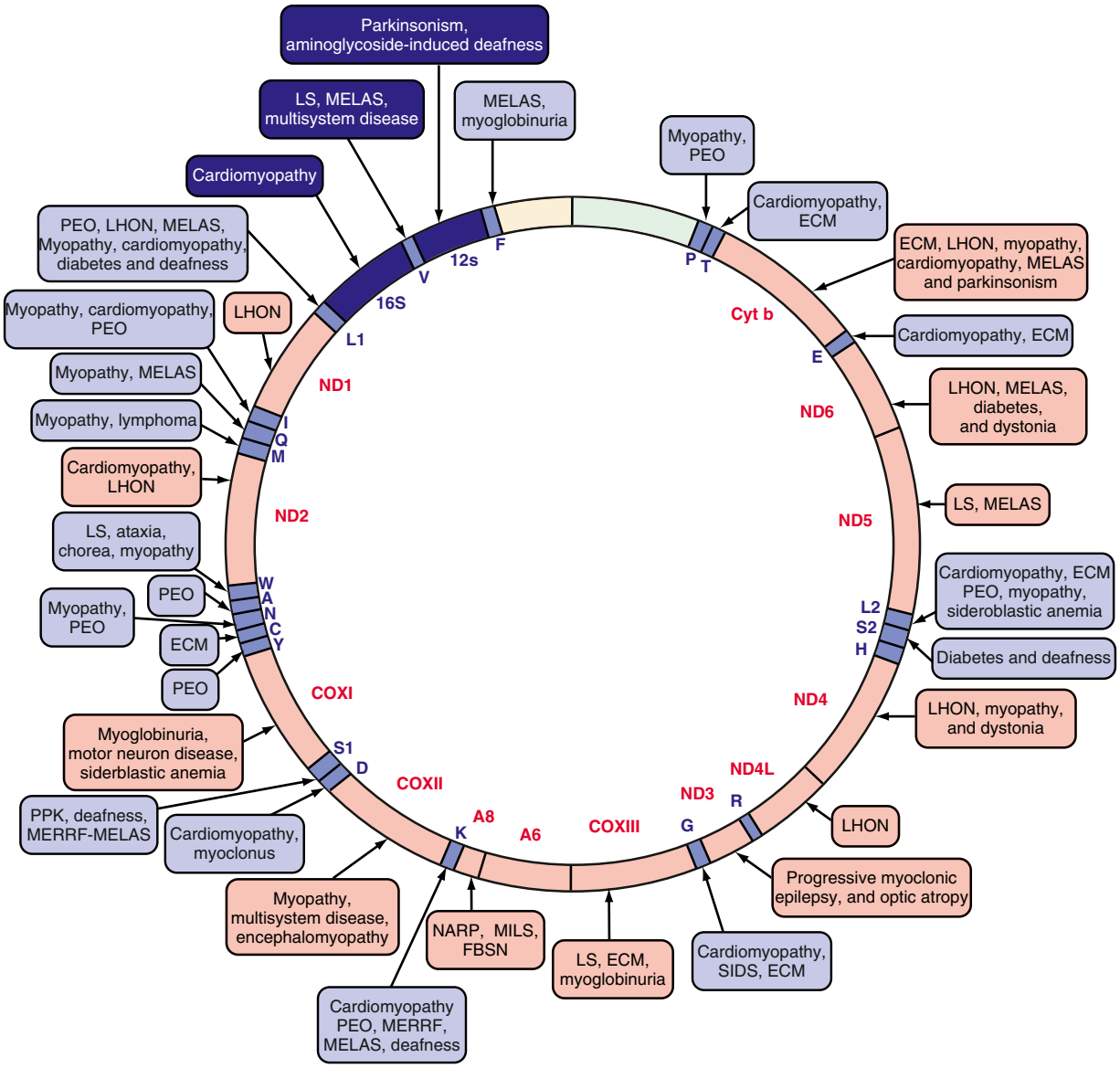

Fig. 107.6 Pathogenic variants in the human mitochondrial genome that are known to cause disease. Disorders that are frequently or prominently associated with pathogenic variants in a particular gene are shown in bold. Diseases caused by pathogenic variants that impair mitochondrial protein synthesis are shown in blue. Diseases caused by pathogenic variants in protein-coding genes are shown in red. ECM, Encephalomyopathy; FBSN, familial bilateral striatal necrosis; LHON, Leber hereditary optic neuropathy; LS, Leigh syndrome; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes; MERRF, myoclonic epilepsy with ragged red fibers; MILS, maternally inherited Leigh syndrome; NARP, neuropathy, ataxia, and retinitis pigmentosa; PEO, progressive external ophthalmoplegia; PPK, palmoplantar keratoderma; SIDS, sudden infant death syndrome. *(From DiMauro S, Schon EA. Mitochondrial respiratory-chain diseases. N Engl J Med. 2003;348:2656–2668.)*

some instances, combined molecular and functional testing may be needed (Fig. 107.6). *NGS of single or combined mitochondrial DNA and nuclear gene panels is the preferred method of testing.* Whole exome sequencing with analysis of the mitochondrial genome or whole genome sequencing may be considered if NGS panel testing is nondiagnostic. Biopsy and genetic analysis of clinically affected tissues, with or without biochemical testing, is indicated when genetic testing of blood is negative or inconclusive and there is clinical suspicion of mitochondrial disease. Muscle biopsy was considered the most reliable procedure for diagnosing mitochondrial diseases prior to the availability of molecular genetic testing. Muscle histology, including EM, can detect ragged red fibers and other abnormalities typical of mitochondrial myopathies. Analysis of oxidative phosphorylation complexes I-IV from intact mitochondria isolated from fresh skeletal muscle can be helpful for the diagnosis of mitochondrial disorders; however, electron transport chain testing of flash-frozen muscle provides an alternative approach when fresh muscle testing is not available. The presence of normal muscle biochemistry and histology does not rule out the possibility of mitochondrial disease. Several disorders, such as defects in mitochondrial maintenance, fusion/fission, translation, transcription, or abnormalities in mitochondrial membrane integrity or transport, may not manifest with substantial oxidative phosphorylation deficiency and may have normal muscle biochemistry and histology. Specific criteria may assist in making a clinical diagnosis (Table 107.4). One study conducted to reevaluate the clinical utility of mitochondrial disease criteria (MDC) determined that the criteria are still beneficial and can aid in directing the diagnostic workup and in interpreting results and deciding on muscle biopsy in certain instances. Additionally, the study demonstrated that whole exome sequencing might be used to diagnose patients with lower MDC scores as having primary mitochondrial disorders. Table 107.5 lists clues to the diagnosis of mitochondrial diseases.

Numerous nuclear genes involved in mitochondrial function and causative for the majority of mitochondrial disorders are included in

Table 107.4 Mitochondrial Disease Criteria (Simplified Version for Bedside Use)*

I. CLINICAL SIGNS AND SYMPTOMS, 1 POINT/SYMPTOM (max. 4 points)				
A. MUSCULAR PRESENTATION (max. 2 points)	**B. CNS PRESENTATION (max. 2 points)**	**C. MULTISYSTEM DISEASE (max. 3 points)**	**II. METABOLIC/IMAGING STUDIES (max. 4 points)**	**III. MORPHOLOGY (max. 4 points)**
Ophthalmoplegia†	Developmental delay	Hematology	Elevated lactate†	Ragged red/blue fibers‡
Facies myopathica	Loss of skills	GI tract	Elevated L/P ratio	COX-negative fibers‡
Exercise intolerance	Stroke-like episode	Endocrine/growth	Elevated alanine†	Reduced COX staining‡
Muscle weakness	Migraine	Heart	Elevated CSF lactate†	Reduced SDH staining
Rhabdomyolysis	Seizures	Kidney	Elevated CSF protein	SDH positive blood vessels†
Abnormal EMG	Myoclonus	Vision	Elevated CSF alanine†	Abnormal mitochondria/EM†
	Cortical blindness	Hearing	Urinary TA excretion†	
	Pyramidal signs	Neuropathy	Ethylmalonic aciduria	
	Extrapyramidal signs	Recurrent/familial	Strokelike picture/MRI	
	Brainstem involvement		Leigh syndrome/MRI†	
			Elevated lactate/MRS	

*Score 1: unlikely mitochondrial disorder; score 2-4: possible mitochondrial disorder; score 5-7: probable mitochondrial disorder; score ≥8: definite mitochondrial disorder.
†This specific symptom scores 2 points.
‡This symptom in a higher percentage scores 4 points.
GI, Gastrointestinal; L/P, lactate/pyruvate; COX, cytochrome C oxidase; SDH, succinate dehydrogenase; EM, electron microscopy; EMG, electromyography; TA, tricarbon acid.
From Morava E, van den Heuvel L, Hol F, et al. Mitochondrial disease criteria – diagnostic applications in children. *Neurology*. 2006;67:1823–1826.

clinical testing panels that are used to diagnose mitochondrial disorders. An important consideration is that many genetic and multifactorial conditions have been associated with defects in one or more of the four complexes assayed in mitochondrial oxidative phosphorylation testing; these latter conditions feature so-called secondary mitochondrial dysfunction because the conditions are not considered to be causative for primary mitochondrial dysfunction.

Treatment remains largely symptomatic and nonspecific and does not cure or significantly alter the outcome of disease. Mitochondrial drugs are mainly medicinal/nutritional supplements designed to sustain and boost the mitochondrial residual oxidative phosphorylation function, while also reducing oxidative stress. Although there is no consensus on which vitamins and cofactors should be used to treat mitochondrial disease, the main supplementations used are CoQ10, L-carnitine, creatine, alpha-lipoic acid (ALA), and B vitamins. Additional medications/supplements are indicated based on the genetic diagnosis and clinical symptoms of the patient. For example, arginine (IV, oral) can be prescribed in patients with MELAS for treatment and prevention of metabolic strokes. Citrulline and taurine have also been used with some success in ongoing clinical trials and case reports demonstrating promising results. Patients with mitochondrial disease should be encouraged to exercise regularly. Patients with mitochondrial disorders are at a greater risk of developing anesthesia-related complications. Avoiding prolonged fasting and receiving dextrose-containing IV fluids before, during, and after procedures and operations are critical for avoiding catabolism. There are an increasing number of clinical studies evaluating various therapeutic approaches, including mitochondrial augmentation therapy in pediatric children with Pearson syndrome (NCT03384420) and AAV gene therapy in individuals with Leber hereditary optic neuropathy (LHON) (NCT02161380).

Leigh Syndrome (Subacute Necrotizing Encephalomyelopathy)

Leigh syndrome is a heterogeneous neurologic disease historically diagnosed based on neuropathologic findings of demyelination, gliosis, necrosis, relative neuronal sparing, and capillary proliferation in specific brain regions (see Chapter 638.2). Leigh syndrome is characterized by decompensation (neurodevelopmental regression and lactic acidosis) initiated by viral infection or other metabolic stress. Patients frequently present with feeding and swallowing problems, failure to thrive, and developmental delay. The presentation is highly variable and may include seizures, altered consciousness, movement disorders, nystagmus, ophthalmoplegia, cerebellar ataxia, and peripheral neuropathy. Extraneurologic manifestations may include pericardial effusion, cardiomyopathy, renal tubulopathy, liver involvement, hypertrichosis, and muscle weakness. **Diagnosis** is usually confirmed by radiologic evidence of symmetric lesions affecting the basal ganglia, brainstem, and subthalamic nuclei. Patients with Leigh syndrome have defects in several enzyme complexes. Dysfunction in cytochrome-c oxidase (complex IV) is the most commonly reported defect, followed by NADH–coenzyme Q reductase (complex I), PDHC, and pyruvate carboxylase (see Chapter 108). Pathogenic variants in the nuclear *SURF1* gene, which encodes an assembly factor involved in the biogenesis of cytochrome-c oxidase, and mitochondrial DNA variants in the adenosine triphosphatase 6 (MT-ATP6) coding region have been reported in patients with Leigh syndrome in association with complex IV deficiency. The most common mitochondrial DNA variant in Leigh syndrome is the m.8993T>G variant in MT-ATP6. The **prognosis** for Leigh syndrome is poor. In a study of 14 cases, there were 7 fatalities before age 1.5 years. Pathogenic variants in *MTFMT* had a milder clinical phenotype and disease progression, according to recent research.

Lactic acidosis and encephalopathy have also been reported in patients with thiamine transporter 2 (THTR2) deficiency and with pyridoxine-dependent epilepsy. Thiamine, either alone or in combination with biotin, is required for the treatment of THTR2 deficiency, whereas pyridoxine is used to treat pyridoxine-dependent epilepsy.

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107.5 Defects in Pentose Metabolism

Ghada Hijazi and Priya S. Kishnani

Approximately 90% of glucose metabolism in the body is generated via the glycolytic pathway, with the remaining 10% generated via the hexose monophosphate pathway. The hexose monophosphate shunt leads to the formation of pentose and provides NADPH. One of the metabolites of this alternative pathway is ribose-5-phosphate, which is used in the biosynthesis of ribonucleotides and deoxyribonucleotides. Through the transketolase and transaldolase reactions, pentose phosphates can be converted back to fructose-6-phosphate and glucose-6-phosphate.

ESSENTIAL PENTOSURIA

Essential pentosuria is a benign asymptomatic disorder encountered principally in the Ashkenazi Jewish population and is inherited in an

Table 107.5 Clues to the Diagnosis of Mitochondrial Disease

NEUROLOGIC
Cerebral strokelike lesions in a nonvascular pattern
Basal ganglia disease
Encephalopathy, recurrent or with low/moderate dosing of valproate
Neurodegeneration
Epilepsia partialis continua
Myoclonus
Ataxia
MRI findings consistent with Leigh syndrome
Characteristic MRS peaks
Lactate peak at 1.3 ppm TE (time to echo) at 35 and 135
Succinate peak at 2.4 ppm
CARDIOVASCULAR
Hypertrophic cardiomyopathy with rhythm disturbance
Unexplained heart block in a child
Cardiomyopathy with lactic acidosis (>5 mM)
Dilated cardiomyopathy with muscle weakness
Wolff-Parkinson-White arrhythmia
OPHTHALMOLOGIC
Retinal degeneration with signs of night blindness, color vision deficits, decreased visual acuity, or pigmentary retinopathy
Ophthalmoplegia/paresis
Fluctuating, dysconjugate eye movements
Ptosis
Sudden- or insidious-onset optic neuropathy/atrophy
GASTROENTEROLOGIC
Unexplained or valproate-induced liver failure
Severe dysmotility
Pseudoobstructive episodes
OTHER
A newborn, infant, or young child with unexplained hypotonia, weakness, failure to thrive, and a metabolic acidosis (particularly lactic acidosis)
Exercise intolerance that is not in proportion to weakness
Hypersensitivity to general anesthesia
Episodes of acute rhabdomyolysis
Elevated GDF-15 level

MRI, Magnetic resonance imaging, MRS, magnetic resonance spectroscopy; GDF, growth and differentiation factor.
From Haas RH, Parikh S, Falk MJ, et al. Mitochondrial disease: a practical approach for primary care physicians. *Pediatrics*. 2007;120:1326–1333, Table 1.

autosomal recessive fashion. The urine contains **L-xylulose**, which is excreted in increased amounts because of a block in the conversion of L-xylulose to xylitol as a result of **xylitol dehydrogenase deficiency**. The condition is usually discovered incidentally in a urine test for reducing substances. No treatment is required.

TRANSALDOLASE DEFICIENCY

Transaldolase deficiency is a rare autosomal recessive inborn error of the pentose phosphate pathway. It is more common in Middle Eastern countries and can manifest in three distinct phenotypes. Prenatal intrauterine growth restriction (IUGR), oligohydramnios, and/or hydrops fetalis occur with the most severe phenotype. The second is a neonatal phenotype, where patients may have dysmorphic facial features (triangular-shaped face, low-set ears, wide mouth, and thin lips), cardiovascular abnormalities (ventricular and atrial septal defects), anemia, thrombocytopenia, and hepatosplenomegaly noted in the newborn period. In some cases, hepatic dysfunction was described associated with fibrosis and/or cirrhosis, and transplantation of the liver was indicated in a number of patients. Endocrine abnormalities (abnormal genitalia, vitamin D insufficiency, hypergonadotrophic hypogonadism), renal tubulopathy, and skin abnormalities (cutis laxa, wrinkled skin, capillary hemangioma) have been observed. A third, milder phenotype is described as presenting later in life. Biochemical abnormalities reveal elevated levels of the polyols arabitol, ribitol, and erythritol and the seven-carbon sugars sedoheptitol, perseitol, sedoheptulose, mannoheptulose, and sedoheptulose-7P in the urine. The **diagnosis** is established with the detection of biallelic pathogenic variants in the *TALDO1* gene. When genetic testing is inconclusive, low transaldolase activity in lymphoblasts, fibroblasts, and liver tissue can be used to confirm the diagnosis. The available **treatment** is symptomatic. Supportive management of the disease manifestations (e.g., hepatic dysfunction) is needed. Transplantation of the liver may be indicated. Regular follow-up and monitoring for complications such as anemia and thrombocytopenia is required. N-acetylcysteine (NAC) supplementation delayed the progression of disease and restored normal alpha fetoprotein levels in a research trial. Additional long-term studies are required to evaluate the broader effectiveness of NAC supplementation.

RIBOSE-5-PHOSPHATE ISOMERASE DEFICIENCY

Only four cases of ribose-5-phosphatase isomerase deficiency disorder have been reported. Affected patients may present with psychomotor delay or regression, epilepsy, peripheral neuropathy, and leukoencephalopathy. Magnetic resonance spectroscopy (MRS) and urine analysis usually reveal elevated levels of polyols arabitol and ribitol in these patients. Confirmation of diagnosis is established by identification of biallelic *RPIA* pathogenic variants or by enzyme assay in cultured fibroblasts.

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107.6 Disorders of Glycoprotein Degradation and Structure

Margaret M. McGovern and Robert J. Desnick

The disorders of glycoprotein degradation and structure include several lysosomal storage diseases that result from defects in glycoprotein degradation and the congenital disorders of glycosylation (see Chapter 107.7). *Glycoproteins* are macromolecules composed of oligosaccharide chains linked to a peptide backbone. They are synthesized by two pathways: the glycosyltransferase pathway, which synthesizes oligosaccharides linked *O*-glycosidically to serine or threonine residues, and the dolichol, lipid-linked pathway, which synthesizes oligosaccharides linked N-glycosidically to asparagine.

The **glycoprotein lysosomal storage diseases** result from the deficiency of the enzymes that normally participate in the degradation of oligosaccharides and include sialidosis, galactosialidosis, aspartylglucosaminuria, and α-mannosidosis. In some instances, the underlying abnormality that leads to glycoprotein accumulation also results in abnormal degradation of other classes of macromolecules that contain similar oligosaccharide linkages, such as certain glycolipids and proteoglycans. In these cases the underlying enzymatic deficiency results in the accumulation of both glycoproteins and *glycolipids*. The classification of these types of disorders as *lipidoses* or *glycoproteinoses* depends on the nature of the predominantly stored substance. In general, the glycoprotein disorders are characterized by autosomal recessive inheritance and a progressive disease course with clinical features that resemble those seen in the mucopolysaccharidoses.

SIALIDOSIS AND GALACTOSIALIDOSIS

Sialidosis is an autosomal recessive disorder that results from the primary deficiency of neuraminidase because of pathogenic variants in the gene *(NEU1)* that encodes this protein. In contrast, **galactosialidosis** is caused by the deficiency of two lysosomal enzymes—neuraminidase and β-galactosidase. The loss of these enzymatic activities results from pathogenic variants in a single gene, *CTSA,* that encodes the

protective protein cathepsin A, which functions to stabilize these enzymatic activities. Neuraminidase normally cleaves terminal sialyl linkages of several oligosaccharides and glycoproteins. Its deficiency results in the accumulation of oligosaccharides and the urinary excretion of sialic acid terminal oligosaccharides and sialylglycopeptides. Examination of tissues from affected individuals reveals pathologic storage of substrate in many tissues, including liver, bone marrow, and brain.

The clinical phenotype associated with neuraminidase deficiency is variable and includes **type I** sialidosis, which usually presents in the second decade of life with myoclonus and cherry-red spots in the macula. These patients typically present secondary to gait disturbances, myoclonus, or visual complaints. In contrast, **type II** sialidosis occurs at several ages of onset (congenital, infantile, and juvenile), depending on the severity of the gene pathogenic variant. The **congenital** and **infantile** forms result from isolated neuraminidase deficiency, whereas the **juvenile** form results from both neuraminidase and β-galactosidase deficiency. The congenital type II disease is characterized by hydrops fetalis, neonatal ascites, hepatosplenomegaly, stippling of the epiphyses, periosteal cloaking, and stillbirth or death in infancy. The type II infantile form presents in the first years of life with dysostosis multiplex, moderate global developmental delays, visceromegaly, corneal clouding, cherry-red maculae, and seizures. The juvenile type II form of sialidosis, which is sometimes designated *galactosialidosis,* has a variable age of onset ranging from infancy to adulthood. In infancy, the phenotype is similar to that of GM1 gangliosidosis, with edema, ascites, skeletal dysplasia, and cherry-red spots. Patients with later-onset disease have dysostosis multiplex, visceromegaly, intellectual disability, dysmorphism, corneal clouding, progressive neurologic deterioration, and cherry-red spots.

No specific therapy exists for any form of the disease, although studies in animal models have demonstrated improvement in the phenotype after bone marrow transplantation. The **diagnosis** of sialidosis and galactosialidosis is achieved by the demonstration of the specific enzymatic deficiency or by pathogenic variants in the responsible gene. **Prenatal diagnosis** using cultured amniotic cells or chorionic villi is available by demonstrating the enzyme defect and/or specific gene pathogenic variants.

ASPARTYLGLUCOSAMINURIA

This is a rare autosomal recessive lysosomal storage disorder, except in Finland, where the carrier frequency is estimated at 1 in 36 adults, the high frequency due to a *founder gene.* The disorder results from the deficient activity of **aspartylglycosaminidase** and the subsequent accumulation of aspartylglycosamine, particularly in the liver, spleen, and thyroid. The gene for the enzyme is *AGA,* and in the Finnish population, a single *AGA* pathogenic variant encoding p.Cys163Ser accounts for most mutant alleles. Outside of Finland, a large number of private pathogenic variants have been described.

Affected individuals with aspartylglucosaminuria typically present in the first year of life with recurrent infections, diarrhea, and umbilical hernias. Coarsening of the facies and short stature usually develop later. Other features include joint laxity, macroglossia, hoarse voice, crystal-like lens opacities, hypotonia, and spasticity. Psychomotor development is usually near normal until age 5 years, when a decline is noted. Behavioral abnormalities are typically seen, and IQ values in affected adults are usually <40 (severe intellectual disability). Survival to adulthood is common, with most early deaths attributable to pneumonia or other pulmonary causes. Definitive **diagnosis** requires demonstration of markedly deficient aspartylglucosaminidase in peripheral blood leukocytes and/or the specific *AGA* pathogenic variant(s). Several patients have undergone allogeneic bone marrow transplants, but this approach has not proved effective, and no specific treatment is available. **Prenatal diagnosis** is available by the determination of aspartylglucosaminidase deficiency and/or the specific *AGA* pathogenic variants in cultured amniocytes or chorionic villi.

α-MANNOSIDOSIS

This autosomal recessive disorder results from the deficient activity of **α-mannosidase** and the accumulation of mannose-rich compounds. The gene *MAN2B1* encodes the enzyme, and to date, >140 gene pathogenic variants have been reported. Affected patients display clinical heterogeneity. There is a severe infantile form, or **type I** disease, and a milder juvenile variant, **type II** disease. All patients have psychomotor retardation, facial coarsening, and dysostosis multiplex. The **infantile** form of the disorder, however, is characterized by more rapid cognitive deterioration, with death occurring between ages 3 and 10 years. Patients with the infantile form also have more severe skeletal involvement and hepatosplenomegaly. The **juvenile** disorder is characterized by onset of symptoms in early childhood or adolescence, with milder somatic features and survival to adulthood. Hearing loss, destructive synovitis, pancytopenia, and spastic paraplegia have been reported in type II patients. The **diagnosis** is made by identification of biallelic pathogenic variants in *MAN2B1* and may be supplemented by the demonstration of the marked deficiency of α-mannosidase activity in white blood cells or cultured fibroblasts. Clinical trials of ERT with recombinant human α-mannosidase are underway. **Prenatal diagnosis** can be made by demonstrating the enzyme defect and/or known pathogenic variants in cultured amniocytes or chorionic villi.

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107.7 Congenital Disorders of Glycosylation

Eva Morava and Peter Witters

Glycosylation is the complex multistep metabolic process of adding (oligo)saccharides to macromolecules such as proteins and lipids. The classification of **disorders of hypoglycosylation** is based on biochemical structures: (1) defects in protein *N*-linked glycosylation, (2) defects in protein *O*-linked glycosylation, (3) defects in glycosphingolipid and in glycosylphosphatidylinositol-anchor glycosylation, and (4) defects in multiple glycosylation pathways and in other pathways (Fig. 107.7). No disorders are known to result from abnormal *C*-linked glycosylation. Congenital disorders of glycosylation (CDGs) are labeled based on their genetic defect.

Protein glycosylation is an essential process. Most functional proteins are glycosylated, including serum proteins (e.g., transferrin, ceruloplasmin, thyroxine-binding globulin [TBG]), hormones (e.g., thyroid-stimulating hormone [TSH], follicle-stimulating hormone [FSH], luteinizing hormone [LH], adrenocorticotropic hormone [ACTH], IGFBP3), and clotting and anticoagulation factors (e.g., factors IX and XI, antithrombin). Membrane proteins are also highly glycosylated. Important intracellular glycoproteins include enzymes such as glycosyltransferases or lysosomal enzymes.

***N*-glycans** are linked to the amide group of asparagine. They are synthetized in a complicated process throughout the cytoplasm, endoplasmic reticulum (ER), and Golgi complex, starting with sugar activation and nucleotide sugar synthesis, then oligosaccharide assembly, and finally glycan processing (see Fig. 107.7). Most of the pediatric glycosylation disorders are the result of N-glycosylation defects. ***O*-glycans** are linked to the hydroxyl group of serine or threonine. These diverse glycoproteins are mostly formed in the Golgi complex; their defects can involve xylosylation, fucosylation, mannosylation, or other modifications. An important focus is *O*-mannosylation defects because of their relevance for dystroglycanopathies.

Lipid glycosylation is an essential process for the synthesis of ceramide and ganglioside synthesis. **Glycosylphosphatidylinositols** (GPIs) are very special glycolipids that link various proteins to the plasma membrane, as complex lipid-sugar anchors (GPI anchors, see Fig. 107.7).

CDGs are predominantly multisystem diseases caused by more than 150 different genetic defects in glycoprotein and glycolipid glycan synthesis. Most patients described with CDG have N-glycosylation defects, followed by the fastest-growing groups of CDGs, involving multiple glycosylation pathways and dolicholphosphate synthesis. Smaller groups are *O*-glycosylation disorders and disorders of GPI. The "oldest" and most common CDG is PMM2-CDG, in which the

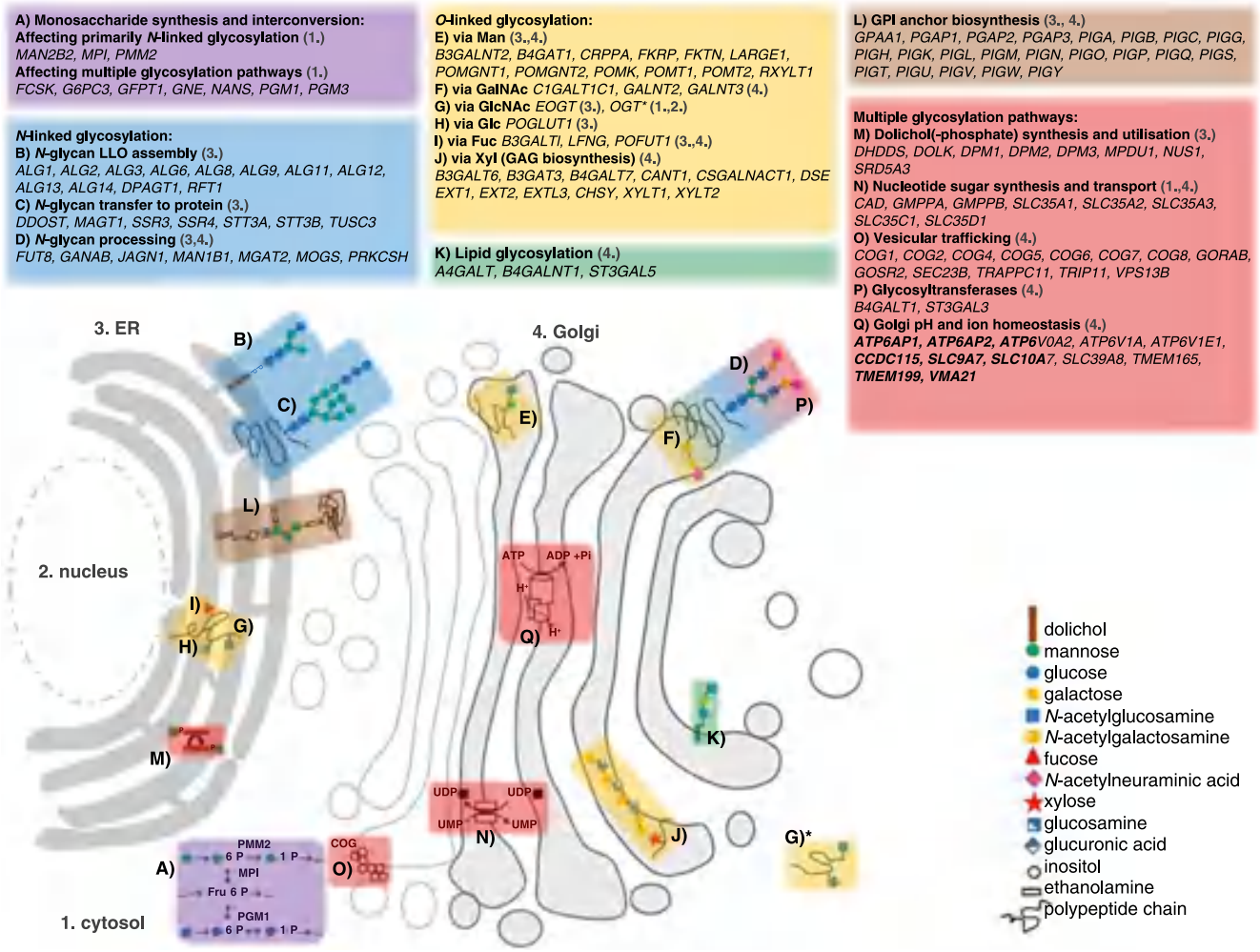

Fig. 107.7 Congenital disorders of glycosylation (CDG): an overview of the currently known subtypes. The picture shows a schematic illustration of the individual 137 CDG subtypes known to date, designated by the symbol of the affected gene, which are categorized according to the nature of the biochemical event impaired within the glycosylation pathway. Glycosylation is a complex metabolic process resulting in the attachment of an oligosaccharide chain of varying length and composition (a glycan) to proteins and lipids. It involves a set of enzyme-catalyzed reactions that take place mainly in the cytosol (1.), endoplasmic reticulum (ER; 3.) and Golgi apparatus (4.), after which the newly formed glycoconjugates are, generally, incorporated into membranes or secreted out of the cell. Depending on how a glycan is linked to the polypeptide backbone, protein glycosylation is classified into *N*-linked (a bond via the amide group of asparagine) or *O*-linked (a bond via the hydroxyl group of serine, threonine, or hydroxylysine). *N*-glycoproteins share a common core structure of the glycan, which is first assembled on a dolichol anchor and then transferred onto the protein in the ER; this is later followed by further modifications of the *N*-glycan, removing and adding different monosaccharides, in the Golgi apparatus. *O*-glycosylation consists of a stepwise addition of individual monosaccharide units in the ER and Golgi apparatus, while six subclasses can be distinguished based on which sugar (Man, GalNac, GlcNAc, Glc, Fuc, or Xyl) is the first one attached to the protein, and thus more varied *O*-glycan structures are produced. Other specific types of glycosylation involve lipid glycosylation and the modification of proteins by the glycosylphosphatidylinositol (GPI) anchor. The substrates for glycosylation in the form of nucleotide-monosaccharides are synthesized predominantly in the cytosol, and because they are used in different glycosylation reactions, their deficiency possibly influences the structure of various glycoconjugates. Similarly, several defects have been described that affect multiple glycosylation pathways, and, often, other cellular processes might be disturbed as well (e.g., because of altered ion homeostasis in the Golgi apparatus). ADP, adenosine diphosphate; ATP, adenosine triphosphate; COG, conserved oligomeric Golgi (complex); ER, endoplasmic reticulum; Fru-6-P, fructose-6-phosphate; Fuc, fucose; GalNAc, N-acetylgalactosamine; Glc, glucose; GlcNAc, N-acetylglucosamine; GPI, glycosylphosphatidylinositol; H+, protons; LLO, lipid-linked oligosaccharides; Man, mannose; MPI, mannose phosphate isomerase; PGM1, phosphoglucomutase 1; PMM2, phosphomannomutase 2; UDP, uridine diphosphate; UMP, uridine monophosphate; Xyl, xylose. *(From Ondruskova N, Cechova A, Hansikova H, et al. Congenital disorders of glycosylation: still "hot" in 2020. Biochim Biophys Acta Gen Subj. 2021;1865[1]:129751, Fig. 1.)*

genetic defect leads to the loss of **phosphomannomutase 2** (PMM2), the enzyme that catalyzes the conversion of mannose-6-phosphate into mannose-1-phosphate. The majority of CDGs have an autosomal recessive inheritance. Only two *N*-linked CDGs are autosomal dominant: GANAB-CDG and PRKCSH-CDG. The dominantly inherited *O*-linked CDGs include EXT1-CDG, H63ST-CDG, and POFUT1-CDG. Some CDGs have both an autosomal dominant and recessive phenotype such as EXT2-CDG, POGLUT1-CDG, GNE-CDG, DHDDS-CDG, NUS1-CDG, COG4-CDG, and SEC23B-CDG. X-linked CDGs include ALG13-CDG, MAGT1-CDG, SSR4-CDG, HS6ST2-CDG, OGT-CDG, PIGA-CDG, SLC35A2-CDG, TRAPPC2-CDG, VMA21-CDG, ATP6AP2-CDG, ATP6AP1-CDG, and SLC9A7-CDG.

CDGs can be lethal, with 20% of PMM2-CDG patients dying in the first 2 years of life. Some patients, however, stabilize throughout

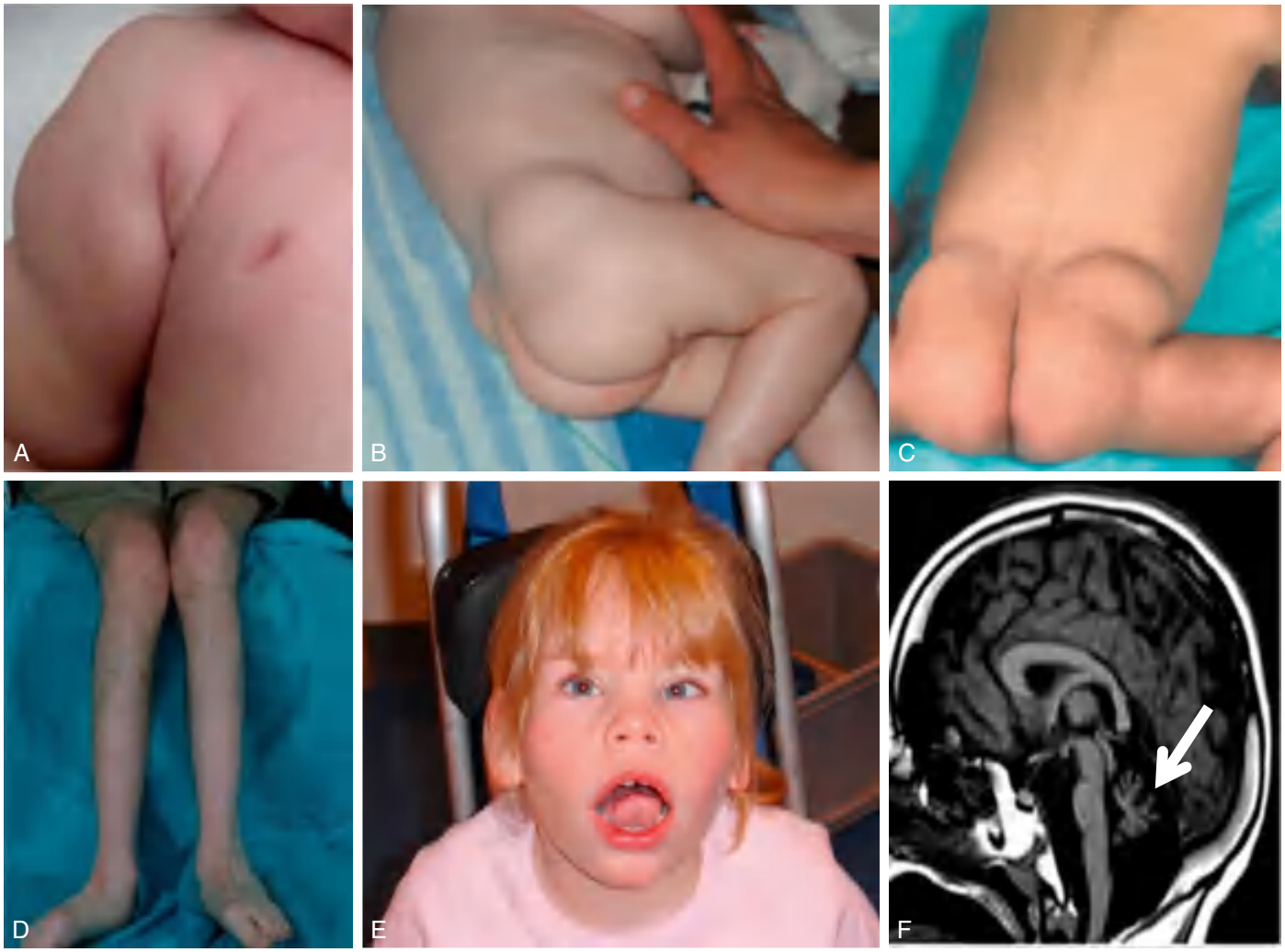

Fig. 107.8 Patients with phosphomannomutase-2 deficiency (PMM2-CDG) and recognizable clinical features. **A,** Inverted nipples. **B** and **C,** Abnormal fat distribution. **D,** Muscle atrophy caused by peripheral neuropathy after puberty. **E,** Characteristic facial features with strabismus, short nose, anteverted nares, long philtrum, and large ears. **F,** T1-weighted sagittal MRI of the brain showing cerebellar vermis hypoplasia *(arrow)* and brain atrophy.

young adulthood. Almost any clinical phenotype can be present in a patient with CDG. It can affect any organ or organ system and most often includes the CNS. The most **common clinical features** include developmental and speech delay, seizures, ataxia, spasticity, peripheral neuropathy, hypotonia, strabismus, abnormal fat distribution, visual loss, cardiomyopathy, feeding difficulties, liver dysfunction, endocrine abnormalities, bleeding diathesis, and thrombosis (Fig. 107.8 and Table 107.6). Single-organ presentations are rare in CDGs, although some do exist, including TUSC3-CDG and ST3GAL3-CDG: brain; DHDDS-CDG: retina; ALG14-CDG: neuromuscular junction; POFUT1-CDG and POGLUT1-CDG: skin; SEC23B-CDG: red cell lineage; EXT1/EXT2-CDG: cartilage; and TMEM199-CDG: liver. Many CDGs are recognizable syndromes. *CDG should be considered in any patient with a developmental disability or an unexplained clinical condition, especially in multisystem disease with neurologic involvement.*

There are also **congenital disorders of deglycosylation**, including known lysosomal disorders and a severe neurologic condition caused by a defective *N*-glycanase function (*NGLY1* defect).

Laboratory evaluations in most *N*-linked CDGs rely on transferrin glycoform analysis in serum or plasma. A common screening method is called serum **transferrin isoelectric focusing (TIEF)**. Transferrin isoforms, which are hyposialylated (missing terminal sialic acid residues), show different cathodal shifts depending on either missing glycan chains or truncated glycans. A **type 1 pattern** suggests an early metabolic defect in the cytosolic-ER–related glycan synthesis and assembly. A **type 2 pattern** suggests Golgi-related glycan-processing defects (Fig. 107.9). **Isoelectric focusing of apolipoprotein C-III (IEF apoC-III)**, a serum mucine type *O*-glycosylated protein, can detect some *O*-glycosylation disorders (combined *N*- and *O*-linked glycosylation defects). **Mass spectrometry of intact (glycosylated) transferrin in serum** is highly sensitive for mild type 1 glycosylation abnormalities and diagnostic in most CDGs affecting N-glycosylation. Mass spectrometry of apoC-III is useful specifically in type II CDG. Glycomics by **matrix-assisted laser desorption/ionization time of flight (MALDI-TOF)** can be diagnostic in specific types of CDG (mostly Golgi related with a type 2 pattern). Dolichol-linked glycan or lipid-linked oligosaccharide (LLO) analysis is a complicated but sensitive method to detect ER-related N-glycan assembly (CDG type 1) defects in patient fibroblasts. GPI-anchor defects can be suspected based on *recurrent elevation of alkaline phosphatase levels* in blood.

Table 107.6 Clinical and Laboratory Features in Common Congenital Disorders of Glycosylation (CDGs), with Clinically Recognizable Phenotype and Abnormal Glycosylation, Detectable by Serum Transferrin Isoform Analysis (TIEF)

DEFECTIVE GENE	MOST FREQUENT CLINICAL FEATURES	SUGGESTIVE FEATURES	LABORATORY ABNORMALITIES	OTHER BIOCHEMICAL ANOMALIES
PMM2	Strabismus, nystagmus, smooth philtrum, large ears, vomiting, diarrhea, FTT, axial hypotonia, cerebellar vermis hypoplasia, ataxia, psychomotor disability, seizures, spasticity, neuropathy, pigmentary retinitis	Inverted nipples and/or abnormal fat pads, strokelike episodes	Elevated serum transaminases; hypoalbuminemia, decreased factor IX, XI, and AT activity; low serum ceruloplasmin and TBG levels	Type 1 serum TIEF, decreased PMM activity in leukocytes and fibroblasts
MPI	Cholestasis, hepatomegaly, feeding difficulties, recurrent vomiting, chronic diarrhea, ascites, recurrent thrombosis, gastrointestinal bleeding	Hyperinsulinism, protein-losing enteropathy Normal intelligence and absence of neurologic features	Elevated transaminases; hypoalbuminemia; hypoglycemia; decreased factor IX, XI, and AT-III activity	Type 1 serum TIEF, decreased PMI activity in leukocytes and fibroblasts
ALG6	Hypotonia, muscle weakness, seizures, ataxia, intellectual disability, behavioral abnormalities	Distal limb malformations	Elevated serum transaminases; hypoalbuminemia; decreased factor IX, XI, and AT activity; low serum IgG level	Type 1 serum TIEF, abnormal LLO results in fibroblasts
DPAGT1	Microcephaly, brain malformations, hypotonia, severe psychomotor disability, seizures, spasticity, proximal weakness, failure to thrive, joint contractures	Congenital myasthenia phenotype In multisystem phenotype: cataract	Decreased AT, protein C, and protein S activity; increased creatine kinase; hypoalbuminemia; normal creatine kinase in myasthenia	Type 1 serum TIEF
SRD5A3	Developmental delay, hypotonia, ataxia, cerebellar vermis hypoplasia, intellectual disability, speech delay, visual loss	Congenital cataract, retinal and iridic coloboma, glaucoma, optic nerve dysplasia, ichthyosis	Low anticoagulation factors (AT, protein C, and protein S activity), increased serum transaminases	Type 1 serum TIEF but reported false-negative TIEF
ATP6V0A2 *ATP6V1A* and *ATP6V1E1*	Generalized cutis laxa, hypotonia, strabismus, characteristic facial features, joint laxity, seizures, motor and language developmental delay, spontaneous improvement of cutis laxa by aging	Cobblestone-like brain dysgenesis Cardiovascular anomalies	Mild coagulation abnormalities, increased serum transaminase levels Mild coagulation abnormalities and increased serum transaminase levels, hypercholesterolemia	Type 2 serum TIEF but reported false-negative TIEF Abnormal apoC-III IEF, characteristic MALDI TOF profile (Note abnormal skin histology)
PGM1	Pierre Robin sequence, cholestasis, short stature, dilated cardiomyopathy	Cleft palate, hyperinsulinism, normal intelligence	Hypoglycemia, increased serum transaminase levels, decreased AT	Mixed type 1/ serum TIEF, decreased fibroblast PGM1 activity
MAN1B1	Developmental delay, speech delay, intellectual disability, muscle weakness	Obesity, autistic features, inverted nipples, characteristic face	Increased serum transaminase levels, low AT	Type 2 serum TIEF, abnormal apoC-III IEF, diagnostic MALDI TOF profile
TMEM199 *CCDC115* *ATP6AP1* and *ATP6AP2*	Cholestasis, hepatomegaly, liver steatosis, liver fibrosis, liver failure, spontaneous bleedings, motor developmental delay	Normal intelligence Hepatomegaly Immune deficiency	Decreased serum ceruloplasmin, increased serum transaminase levels, hypercholesterolemia, high AP	Type 2 serum TIEF, abnormal apoC-III IEF, characteristic MALDI TOF profile
SLC39A8	Seizures, hypsarrhythmia, hypotonia, developmental and speech delay, FTT	Dwarfism, craniosynostosis, rhizomelia, Leigh disease	Decreased serum manganese, high serum transaminases, abnormal coagulation	Type 2 serum TIEF, abnormal apoC-III, characteristic MALDI TOF profile

AP, Alkaline phosphatase; AT, antithrombin; apoC-III: apolipoprotein C-III; FTT, failure to thrive; LLO, lipid-linked oligosaccharides; MALDI-TOF, matrix-assisted laser desorption/ionization time of flight; TBG, thyroxine-binding globulin; TIEF, transferrin isoelectric focusing.

Fluorescence-activated cell sorting (FACS) analysis of the membrane-anchored markers CD16 and CD24 in leukocytes is highly suggestive for a GPI-anchor abnormality, especially when alkaline phosphatase in the blood is significantly elevated. **Enzyme analysis** in blood is only available for a few, more common CDGs (PMM2-CDG, MPI-CDG, PGM1-CDG); it is more reliable in fibroblasts. Dystroglycanopathies can be confirmed based on abnormal immunohistochemistry in a **muscle biopsy**.

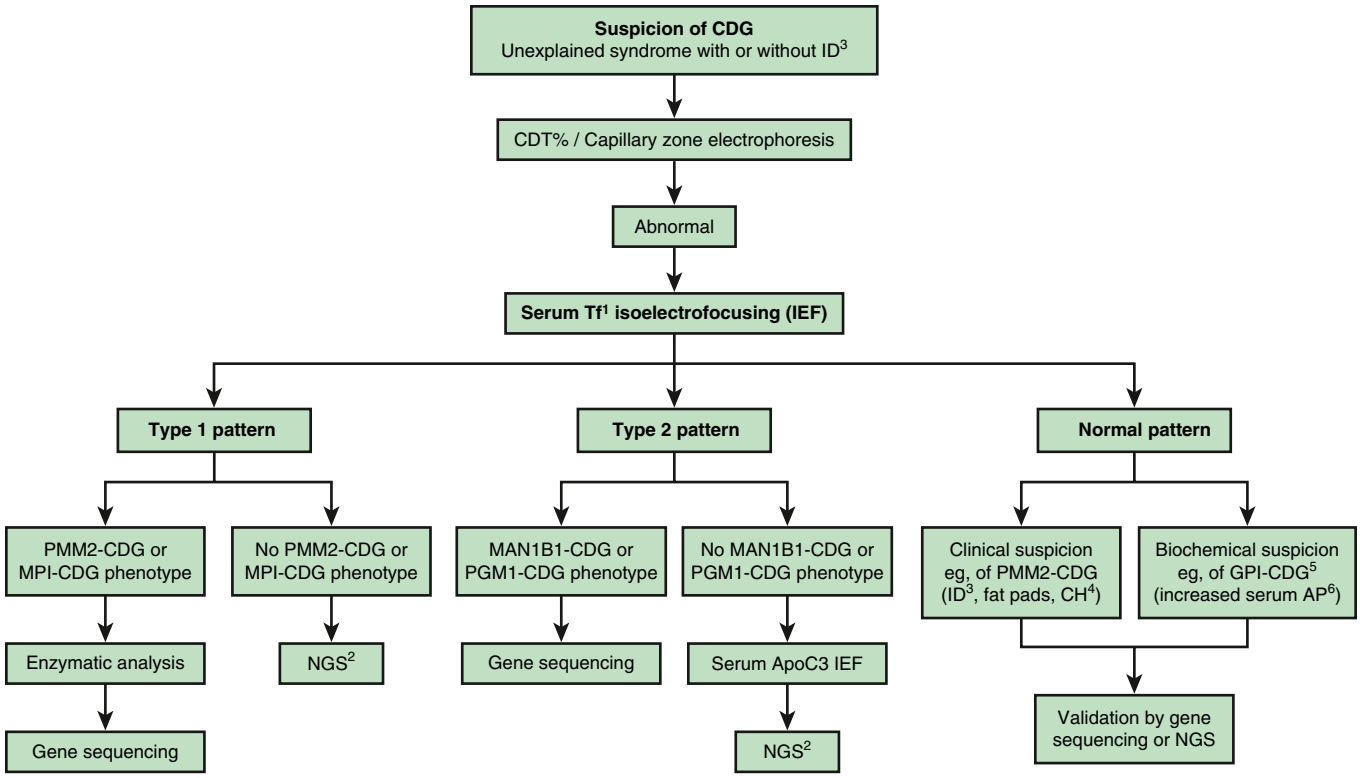

Fig. 107.9 CDG diagnosis algorithm. [1]Transferrin; [2]Next-generation sequencing (CDG panel analysis, WES, WGS); [3]Intellectual disability; [4]Cerebellar hypoplasia; [5]Glycosylphosphatidylinositol anchor synthesis defect; [6]Alkaline phosphatase. ID, intellectual disability; WES, whole exome sequencing; WGS, whole genome sequencing. *(From Quelhas D, Martins E, Azevedo L, et al. Congenital disorders of glycosylation in Portugal – two decades of experience. J Pediatr. 2021;231:148–156.)*

With an abnormal TIEF pattern result or clinical suspicion of any type of CDG, most metabolic centers use a direct **CDG gene panel analysis** or **NGS** (whole exome sequencing) (see Fig. 107.9).

CONGENITAL DISORDERS OF PROTEIN N-GLYCOSYLATION

Phosphomannomutase-2 Deficiency (PMM2-CDG)

Clinical Manifestations

PMM2-CDG is the most common and easily recognizable CDG. Over 900 patients have been identified with an estimated incidence of 1:20,000-1:80,000. Most patients have alternating strabismus, characteristic facial features (short nose, long philtrum, large ears) (see Fig. 107.8E), inverted nipples and/or abnormal fat pads (see Fig. 107.8A-C), feeding difficulties, axial hypotonia, and decreased reflexes already in the first few months of life. Nystagmus (caused by pontocerebellar and vermis hypoplasia; see Fig. 107.8F) is also common. Psychomotor disability is present in most patients, but normal intellectual development has been described in a few patients. Most patients develop a **multisystem disease**, and <25% show an isolated neurologic phenotype without other organ involvement, normal endocrine regulation, and no coagulopathy. The **neurologic involvement** is quite diverse, with ataxia, seizures, spasticity, and peripheral neuropathy (see Fig. 107.8D) the most common features. Dystonia, strokelike episodes, and proximal myopathy can also occur. PMM2-CDG is not a progressive disease, but certain features, when present, typically appear at a different age during the disease. From birth, pericardial fluid collection, cardiomyopathy, or chronic vomiting/diarrhea can occur; after the age of 7 years, retinitis pigmentosa and cataracts; and after puberty, scoliosis, neuropathy, and recurrent thrombotic events. Liver function anomalies are mild and usually improve by age, and only a few patients develop cholestasis or liver fibrosis. Most patients have a hypergonadotropic hypogonadism; no successful pregnancies have been reported. Intellectual disability can be mild to severe; speech development is frequently delayed and can even be absent. Autistic behavior is common, although most patients have a cheerful personality. The oldest patient is 72 years old.

Pathophysiology

PMM2 catalyzes the conversion of mannose-6-phosphate to mannose-1-phosphate and is essential for the formation of activated mannose units used in the synthesis of the growing glycan chain in the ER. Hypoglycosylation leads to abnormal function affecting many essential glycoproteins, such as coagulation and anticoagulation factors; endocrine regulation; transport proteins; liver function; and immune, membrane, and receptor proteins.

Diagnosis

The primary screening method for PMM2-CDG is **serum transferrin glycoform analysis**, which is most frequently performed by TIEF or mass spectrometry. Intact transferrin has four negatively charged sialic acid residues (tetrasialotransferrin). Transferrin glycoforms, missing terminal sialic acid residues, show different cathodal shifts, less abundant tetrasialotransferrin, increased disialotransferrin, and some a-sialotransferrin. This is the so-called type 1 pattern, suggestive of a defect in glycan assembly in the cytosol-ER. Transferrin isoforms are detectable by mass spectrometry, which is a more reliable diagnostic method compared with TIEF. Certain other disorders can cause a *false-positive* transferrin isoform pattern, including galactosemia, hereditary fructose intolerance, and excessive alcohol use. PMM enzyme analysis is available in leukocytes and fibroblasts.

The presence of elevated serum transaminases, hypoalbuminemia, decreased factor IX and XI and antithrombin activity, or low ceruloplasmin or TBG level is highly suggestive of CDG, including the most common type, PMM2-CDG.

PMM2-CDG is autosomal recessive. Genetic testing is mostly performed by direct sequencing. The most frequent pathogenic variant (c.422G>A; R141H) is present in 75% of White patients. Prenatal diagnosis is only reliable by genetic testing.

Treatment

The therapy in PMM2-CDG relies on supportive treatment. Even with the best treatment, mortality is about 20% in the first 2 years of life, mostly from cardiac or kidney involvement and severe infections. Recommended therapy includes adequate nutrition, diet or tube feeding if needed, cardiac support, hormone supplements, physical and occupational therapy, speech therapy, seizure management, and strabismus surgery. Treatment with acetazolamide (7-17 mg/kg/day) resulted in improvement of ataxia and a decrease in disease severity (measured by the Nijmegen Pediatric CDG rating scale). This is likely mediated by the function of the glycosylated CaV2.1 channel. Possible dehydration, changes in plasma pH, and bicarbonate levels (renal tubular acidosis) need to be monitored throughout therapy.

Therapeutic developments include targeted (liposomal) mannose-phosphate treatment, drug repurposing, and chaperone therapy; these are only in early trial phases.

Mannosephosphoisomerase Deficiency (MPI-CDG)

Clinical Manifestations

MPI deficiency is a recognizable and treatable CDG. Approximately 35 patients have been published. Most patients show early symptoms of **liver disease** (cholestasis, elevated transaminases) and feeding difficulties, with recurrent vomiting and chronic diarrhea, most frequently with **protein-losing enteropathy**. Life-threatening episodes might appear as early as the first few months of life with recurrent thrombosis and severe gastrointestinal bleeding because of severe coagulation abnormalities. Hypoglycemia is usually caused by hyperinsulinism. Hypoalbuminemia can be severe; patients might develop visible abdominal distention from a combination of ascites and hepatomegaly. Patients with MPI-CDG have no other organ involvement, and the CNS is not affected. There are no dysmorphic features. The liver disease frequently progresses to fibrosis or cirrhosis.

Pathophysiology

Mannosephosphoisomerase (MPI) catalyzes the conversion of fructose-6-phosphate to mannose-6-phosphate, one step before PMM2, therefore blocking the formation of activated mannose units (GDP mannose) for oligosaccharide synthesis. Hypoglycosylation leads to abnormal glycoprotein function, the same as in PMM2-CDG, especially coagulation and anticoagulation factors, liver function, and hormone receptors.

Diagnosis

The primary screening method in a suspected MPI-CDG patient is **serum transferrin isoform analysis** by mass spectrometry or screening by TIEF (see Fig. 107.9). MPI deficiency leads to a type 1 pattern, as seen in PMM2 deficiency. MPI enzyme analysis is available in leukocytes and fibroblasts. The presence of elevated serum transaminases, hypoalbuminemia, decreased factor IX and XI and antithrombin activity, hyperinsulinism, and nonketotic hypoglycemia are highly suggestive for MPI-CDG.

MPI-CDG is autosomal recessive. Genetic testing is mostly performed by direct sequencing. The exact incidence of MPI-CDG is not known, but it is estimated at 1 in 800,000 in Europe. Prenatal diagnosis is only reliable by genetic testing. Although this is a rare CDG, early diagnosis is imperative because it is treatable.

Treatment

MPI-CDG is the first CDG type treatable by dietary therapy (Table 107.7). Mannose therapy is clinically effective by both IV and oral supplementation. The dose of oral mannose is 150-170 mg/kg/dose for four or five doses per day (maximum dosing 600-1200 mg/kg/day and maximum of six doses per day). IV mannose should be used only in life-threatening conditions when oral intake is not possible. The maximum dose of IV mannose is 1 g/kg/day as a continuous infusion, combined with IV glucose to prevent hypoglycemia. A known side effect is hemolysis. The treatment uses an alternative pathway: mannose can be phosphorylated by hexokinases to mannose 6-phosphate, bypassing the MPI defect. The clinical symptoms improve rapidly, but liver function may further deteriorate. Liver fibrosis and cirrhosis may necessitate liver transplantation, which will resolve the metabolic disease. The oldest patient known with MPI-CDG has survived into their late 30s.

Glucosyltransferase-1 Deficiency (ALG6-CDG)

Clinical Manifestations

ALG6-CDG is the second most common CDG. Most patients have hypotonia, muscle weakness, seizures, and ataxia. No patient with ALG6-CDG has been reported with normal intelligence. Speech delay and nystagmus are common neurologic signs. Brachydactyly, skeletal abnormalities, and transverse limb defects have been observed. Strabismus and characteristic facial dysmorphism are rare (hypertelorism, oval face, short nose). Inverted nipples and/or abnormal fat pads are exceptional in ALG6-CDG.

The most severe ALG6-CDG patients show a multisystem phenotype in the first few months of life, including severe infections, protein-losing enteropathy, hypoalbuminemia, anemia, and failure to thrive. Autistic behavior and mood changes have been observed in several patients. The oldest patient to date is almost 50 years old.

Pathophysiology

The metabolic problem is caused by defective binding of the first of three glucoses to the lipid-linked oligosaccharide in the ER. This glucose binding is essential for attachment of the oligosaccharyltransferase enzyme complex to the newly built oligosaccharide chain and the ability to transfer it to the protein. This leads to protein hypoglycosylation and abnormal glycoprotein function similar to PMM2-CDG and MPI-CDG. Laboratory abnormalities are also similar, including abnormalities in coagulation and anticoagulation factors, liver function, thyroid hormones, and immunoglobulins (IgGs).

Diagnosis

The primary screening method in a suspected ALG6-CDG patient is **serum transferrin glycoform analysis** by mass spectroscopy or TIEF analysis. ALG6 deficiency leads to a type 1 pattern (see Fig. 107.9), as seen in PMM2 and MPI deficiency. There is no available enzyme analysis, although LLOs could be evaluated in patient fibroblasts.

ALG6-CDG is autosomal recessive. Genetic testing is mostly performed by direct sequencing. The most common pathogenic variants are p.A333V and p.I299Del. Prenatal diagnosis is only reliable by genetic testing. The exact incidence of ALG6-CDG is not known.

Treatment

The current therapy in ALG6-CDG relies on supportive treatment. Mortality is about 10% in the first years of life, mostly from protein-losing enteropathy and severe infections.

UDP-GlcNAc:Dol-P-GlcNAc-P Transferase Deficiency (DPAGT1-CDG)

Clinical Manifestations

DPAGT1 deficiency is a recognizable and potentially treatable CDG. About one third of patients show the **congenital myasthenia**

Table 107.7 Effective Therapies in CDG

CDG	TREATMENT	RECOMMENDED DOSE	CLINICAL EFFECTS	SIDE EFFECTS
MPI-CDG	Mannose	600-1200 mg/kg BW/day in 4-6 oral doses	Improvement of digestive symptoms (13/14), coagulopathy (12/12), and hypoglycemia (9/9); no long-term effect on liver symptoms	Abdominal pain and diarrhea (5/10; responding to dose adjustment)
PGM1-CDG	Galactose	1 g/kg/day (500-2500 mg/kg/day; max 50 g/day), divided in up to 6 oral doses per day	Improvement of hepatopathy (15/18), muscle symptoms (14/14), coagulopathy (10/12), hypoglycemia (10/13), and pubertal delay (2/2); minimal effect on growth (1/8), ID (0/2), and cardiologic symptoms (0/2)	Not reported
SLC35C2-CDG	Galactose	1.5 g/kg/day (up to 3 g/kg/day), divided in up to 5 oral doses per day	Improvement of psychomotor development (5/10), growth (n.s.), gastrointestinal symptoms (n.s.), and seizures (3/5)	Not reported
SLC35C1-CDG	Fucose	400 mg/kg/day (up to 1.2 g/kg/day in severe cases), divided into 2-3 oral doses per day	Normalization of neutrophil count (5/5), decreased incidence of severe infections (3/3), improvement of psychomotor development (1/5), no effect on adult form of the disease (ID, ataxia, epilepsy, autism)	Autoimmune neutropenia and hemolysis (responding to dose adjustment)
CAD-CDG	Uridine	100 mg/kg/day in 4 oral doses	Cessation of pharmacoresistant seizures (3/3), significant developmental progress (3/3), resolution of anemia (2/2), and anisopoikilocytosis (3/3)	Not reported
SLC39A8-CDG	Manganese	15-20 mg $MnSO_4$/kg BW/day in 5 oral doses	Improvement of glycosylation (2/2), motor abilities (2/2), epilepsy (1/1), ataxia (1/1), and hearing (1/1)	Not reported, risk of manganism
	Galactose	500-3750 mg/kg BW/day in 5 oral doses	Improvement of glycosylation (2/2), no clinical improvement reported	Not reported
TMEM165-CDG	Galactose	1000 mg/kg/day orally	Improvement of glycosylation (2/2), hepatopathy (2/2), coagulopathy (2/2), and IGF1 levels (2/2) (clinical improvement not mentioned)	Not reported
GFPT1-CDG	Pyridostigmine (cholinesterase inhibitors)	2.5-15 mg/kg/day in 3 oral doses	Improvement of muscle weakness (52/56)	Muscle twitching, depression, and anxiety (2/56)
ALG2-CDG	Pyridostigmine (cholinesterase inhibitors)	Oral, dose n.s.	Improvement of muscle weakness (1/1)	Not reported
ALG14-CDG	Pyridostigmine (cholinesterase inhibitors)	5-8 mg/kg/day in 2-6 oral doses	Improvement of muscle weakness (4/4, in 1 patient the effect was only temporary)	Not reported
PIGM-CDG	Sodium butyrate	60-90 mg/kg/day in 3 oral doses	Effect on seizures (3/3), developmental delay (2/2), and increase in the expression of GPI-linked blood cell surface markers (2/4)	Not reported
PMM2-CDG	Acetazolamide	7-17 mg/kg/day in 2 oral doses	Effect on cerebellar symptoms (20/23), speech (20/23), anxiety (20/23), some coagulation parameters (20/23), stereotypic movements (5/5), and SLE (1/1). No improvement of ID and non-neurologic symptoms.	Acidosis with significant decrease of serum bicarbonate (9/23), asthenia (4/23), and paresthesia (2/23) responding to dose adjustments; risk of urolithiasis, osteopenia

BW, Body weight; GPI, glycosylphosphatidylinositol; ID, intellectual disability; IGF1, insulin-like growth factor 1; n.s., not specified; SLE, strokelike episodes.
Modified from Ondruskova N, Cechova A, Hansikova H, et al. Congenital disorders of glycosylation: still "hot" in 2020. *Biochim Biophys Acta Gen Subj*. 2021;1865(1):129751, Table 2.

phenotype, indistinguishable from other genetic congenital myasthenias. CK levels are normal. These patients have a relatively good prognosis, especially with early myasthenia therapy. The other patients show a multisystem phenotype with microcephaly, brain malformations, hypotonia, severe psychomotor disability, seizures, spasticity, failure to thrive, joint contractures, and cataracts.

Pathophysiology

The DPAGT1 defect leads to very early arrest of glycan synthesis outside the ER membrane by slowing the addition of the second GlcNAc sugar to the phosphorylated dolichol arm. Abnormal receptor glycosylation in the *neuromuscular junction* leads to myasthenia. Hypoglycosylation in the multisystem type leads to

abnormal glycoprotein function similar to PMM2-CDG, especially involving the anticoagulation factors, and interestingly leading to high serum CK (in contrast to the congenital myasthenia phenotype) and hypoalbuminemia.

Diagnosis

The primary screening method is **serum transferrin glycoform analysis** by mass spectroscopy or TIEF analysis. Most patients show a type 1 pattern (see Fig. 107.9), but patients with the congenital myasthenia phenotype can show normal screening. There is no clinically available enzyme analysis.

DPAGT1-CDG is autosomal recessive. Genetic testing is mostly performed by direct sequencing. The exact incidence is not known. Prenatal diagnosis is only reliable by genetic testing. Because of the false-negative TIEF results in several patients with the myasthenic phenotype, congenital myasthenia panel testing is suggested in suspected cases, especially for determining the potential therapy.

Treatment

The congenital myasthenia phenotype is frequently treatable by high-dose pyridostigmine, eventually enhanced with salbutamol and a potassium channel blocker (amifampridine). In the multisystem phenotype of DPAGT1-CDG, treatment is supportive.

CONGENITAL DISORDERS OF PROTEIN *O*-GLYCOSYLATION

Cerebro-Ocular Dysplasia–Muscular Dystrophy and Muscle-Eye-Brain Disease Spectrum (POMT1-CDG, POMT2-CDG, POMGNT1-CDG)

From isolated muscular dystrophy to **Walker Warburg syndrome**, this group of *O*-linked glycosylation disorders presents with severe muscle weakness, congenital eye malformations, and neuronal migration defects. Pachygyria, cobblestone dysgenesis, hydrocephalus, polymicrogyria, heterotopias, and corpus callosum agenesis are variably present. Eye malformations include anophthalmia, microphthalmia, congenital cataract, or colobomas. **Congenital muscular dystrophy** is associated with significant CK elevations. There is severe psychomotor disability.

The underlying metabolic defect is the abnormal synthesis of the *O*-mannosylglycan core, which is essential for the proper glycosylation of α-dystroglycan. The α-dystroglycan is heavily *O*-glycosylated with mannose residues and is expressed in both muscle and brain. Defective mannosylation of α-dystroglycan leads to muscle degeneration and migration defects. Muscle biopsy shows abnormal α-dystroglycan staining on immunohistochemistry.

Transferrin glycoform analysis, including TIEF, is normal in patients with isolated *O*-mannosylation defects. There is also no clinically available enzyme analysis. **Diagnosis** is based on histology (muscle biopsy) and genetic analysis.

POMT1-CDG, POMT2-CDG, and POMGNT1-CDG are the most common autosomal recessive α-dystroglycanopathies. Additional gene defects occur in the pathway; *POMK, FKTN, FKRP, LARGE, B3GALNT2, B4GAT1, DAG1, TMEM5,* and *ISPD* have been described in association with human disease. The exact incidence of α-dystroglycanopathies is not known.

The treatment for α-dystroglycanopathies is supportive.

DEFECTS IN LIPID GLYCOSYLATION AND IN GLYCOSYLPHOSPHATIDYLINOSITOL ANCHOR BIOSYNTHESIS

Hyperphosphatasia–Intellectual Disability Syndromes: PIGA Deficiency (PIGA-CDG)

This clinically recognizable syndrome is an epilepsy syndrome with intellectual disability, hypotonia, dysmorphic facial features, skin anomalies, congenital brain malformations, and behavioral abnormalities, including autism. Other organ malformations, including cardiac and renal defects, have also been reported. *Somatic* pathogenic variants with a *PIGA* defect can also lead to paroxysmal nocturnal hemoglobinuria.

N-acetylglucosamine (GlcNAc) cannot be efficiently transferred to phosphatidylinositol for glycophosphatidylinositol synthesis. Abnormal anchoring of alkaline phosphatase leads to hyperphosphatasemia in the blood and loss of specific surface antigens on blood cells.

Transferrin isoform analysis is normal in GPI-anchor defects. FACS analysis demonstrating reduction of membrane-anchored markers CD16 and CD24 in leukocytes is highly suggestive for a GPI-anchor abnormality, especially in association with increased levels of serum alkaline phosphatase. Pathogenic variant analysis confirms the defect.

PIGA-CDG is X-linked. The exact incidence is not known. A similar phenotype has been described in *PIGO, PIGV, PIGY, PIG, PGAP2,* and *PGAP3* defects.

In PIGA-CDG the treatment is supportive.

DEFECTS IN MULTIPLE GLYCOSYLATION PATHWAYS AND IN OTHER PATHWAYS, INCLUDING DOLICHOLPHOSPHATE BIOSYNTHESIS DEFECTS

Steroid 5α-Reductase Deficiency (SRD5A3-CDG)

Clinical Manifestations

SRD5A3 deficiency is a clinically recognizable CDG, originally described as a **multiple-congenital malformation syndrome**. About 20 patients have been diagnosed at different ages, including one at 45 years of age. Patients have hypotonia, ataxia, and eye abnormalities, including congenital cataracts, retinal and iridic colobomas, glaucoma, optic nerve dysplasia, and visual loss. Cerebellar vermis hypoplasia can be variable. Intellectual disability has been described in all affected patients thus far. About 30% of patients have severe *congenital ichthyosis*. Hypertrichosis and dysmorphic facial features are common, including squared face, high forehead, large ears, and coarsening. Some children with SRD5A3-CDG have a severe autism spectrum disorder. Skeletal abnormalities (scoliosis) and cardiac malformations are less common.

Pathophysiology

SRD5A3 deficiency leads to abnormal dolichol synthesis affecting early glycan synthesis outside the ER membrane and affects *O*-mannosylation and GPI-anchor synthesis. Hypoglycosylation affects anticoagulation factors and leads to increased serum transaminases.

Diagnosis

The primary screening method in a suspected SRD5A3-CDG patient is **serum transferrin glycoform analysis or mass spectroscopy analysis**. Most patients show a type 1 pattern (see Fig. 107.9), but several false-negative cases have been described. There is no clinically available enzyme analysis.

SRD5A3-CDG is autosomal recessive. Genetic testing is mostly performed by direct sequencing. The exact incidence is not known. SRD5A3-CDG treatment is supportive.

Autosomal Recessive Cutis Laxa Type 2 (ARCL-2A or ATP6V0A2-CDG, ATP6V1A-CDG and ATP6V1E1-CDG)

Clinical Manifestations

ATP6V02-CDG is a multiple-malformation syndrome originally described as *cutis laxa syndrome* and recently discovered to be a combined *N*- and *O*-linked glycosylation disorder. Patients show generalized cutis laxa with inelastic, sagging skin at birth, hypotonia, strabismus, myopia, characteristic facial features, and joint laxity. The facial features include hypertelorism, short nose, long philtrum, downslanting palpebral fissures with sagging eyelids, and sagging cheeks. Cardiovascular involvement is rare, and there is variable CNS involvement. Seizures and motor and language

developmental disability are common, but normal intelligence has been described as well. Sensorineural hearing loss is sometimes observed. Some patients have vermis hypoplasia, and several children have been described with cobblestone-like dysgenesis and partial pachygyria on brain MRI. Skeletal abnormalities and short stature are common, as are late-closing fontanels and/or brachydactyly and scoliosis. There is frequently enamel dysplasia. The skin features spontaneously improve with age. **ATP6V1A-CDG** and **ATP6V1E1-CDG** show a highly overlapping phenotype with associated cardiovascular symptoms and hypercholesterolemia.

Pathophysiology

ATP6V0A2 is a membrane subunit of the proton pump of the vesicular adenosine triphosphatase (V-ATPase) complex. Abnormal function of the V-ATPase complex alters the pH gradient in the secretory pathway and affects the maturation and transport of several glycosyltransferases and elastic fibers (e.g., elastin). ATP6V1A and ATP6V1E1 are other complex subunits affecting ATP6V0A2 function and cause secondary ATPase deficiency. Both *N*- and *O*-linked glycosylation are affected. There are mild coagulation abnormalities and high serum transaminase levels in some patients.

Diagnosis

The primary screening method in a suspected ATP6V0A2-CDG patient is **serum transferrin glycoform analysis** by mass spectroscopy or TIEF analysis. Most patients show a type 2 pattern (see Fig. 107.9), but false-negative cases have been described before the age of 6 weeks. **Apolipoprotein III-C** (apoC-III) is a mucin-type secretory glycoprotein that is only *O*-glycosylated. ApoC-III TIEF shows a hypoglycosylation pattern by mass spectroscopy in patients, even when the TIEF is falsely negative. Skin biopsies in patients show classic histologic changes of cutis laxa with diminished, short, abnormal, and fuzzy elastic fibers.

ATP6V0A2-CDG is autosomal recessive. Genetic testing is mostly performed by direct sequencing. The exact incidence is not known. *ATP6V1A* and *ATP6V1E1* defects have been recently described.

Treatment

In autosomal recessive cutis laxa type 2, the treatment is supportive. Fortunately, there is continuous and spontaneous improvement of skin symptoms throughout the disease course, especially in ATP6V0A2-CDG.

Golgi-$\alpha_{1\text{-}2}$ Mannosidase-1 Deficiency (MAN1B1-CDG)

Clinical Manifestations

The *MAN1B1* defect was originally described as an intellectual disability syndrome in association with dysmorphic features. Additional patients were recognized with psychomotor disability, muscle hypotonia, and inverted nipples in association with truncal obesity. The degree of intellectual disability is quite variable. Autistic behaviors, eating disorders, and aggressive behavior are frequent features. More than 30 patients have been reported.

Pathophysiology

MAN1B1 codes for a Golgi mannosidase, which is essential for the final "trimming" of mannose units during the glycan processing in the Golgi apparatus. Hypermannosylation leads to abnormal, truncated glycans and CDG-II. The glycosylation abnormality in serum is relatively mild. Increased serum transaminases and abnormal coagulation are uncommon.

Diagnosis

Most patients show a mild type 2 pattern by TIEF, but false-negative cases have been described. MALDI-TOF analysis shows characteristic, hybrid glycans in serum. In suspected cases, direct sequence analysis is recommended, even if the TIEF is normal.

MAN1B1-CDG is autosomal recessive. The exact incidence is unknown; several adult patients are known.

Treatment

Only supportive treatment is available.

Phosphoglucomutase-1 Deficiency (PGM1-CDG)

Clinical Manifestations

PGM1-CDG is a disorder presenting with midline malformations (cleft palate, Pierre Robin sequence, bifid uvula), liver dysfunction, hypoglycemia, and short stature in almost all patients. *Hypoglycemia* is usually caused by hyperinsulinism in the first years of life. It can resolve with aging; ketotic hypoglycemia has also been observed. Cholestasis, liver fibrosis, and even cirrhosis have been described in a few patients. About one third of patients also show proximal muscle weakness and dilated cardiomyopathy; the latter led to mortality in at least seven reported cases. Other malformations, including cardiac and skeletal anomalies, have also been described. Wound healing is frequently abnormal, and there is a very high risk for bleeding during surgery. Intelligence is normal.

Pathophysiology

Phosphoglucomutase 1 (PGM1) is an essential enzyme for glycogenolysis and glycolysis. It also provides substrates for the nucleotide sugars needed for normal glycosylation. PGM1 regulates the bidirectional conversion of glucose-1-phosphate and glucose-6-phosphate. During fasting it leads to a glycogenosis-like phenotype (also called GSD XIV, MIM 614921). PGM1-CDG affects both the ER- and Golgi-related glycosylation and causes a mixed type 1/type 2 hypoglycosylation pattern. Abnormal serum proteins include coagulation and anticoagulation factors, insulin-like growth factor–binding protein 3 (IGFBP3), TBG, and TSH, in addition to serum transaminases, hypoglycemia, and elevated CK.

Diagnosis

The primary screening method in a suspected PGM1-CDG patient is **serum transferrin glycoform analysis** or mass spectroscopy analysis. Patients show a mixed type 1/type 2 pattern.

PGM1-CDG is autosomal recessive. It is among the relatively common CDGs; >40 patients have been described. Enzyme testing is possible in blood but is more reliable in fibroblasts. Direct sequencing is available for testing.

Treatment

PGM1-CDG is a treatable CDG (see Table 107.1). D-Galactose replenishes depleted levels of different nucleotide-sugars (galactose-1-phosphate, UDP-galactose, and UDP-glucose). Adding 1 g/kg/day D-galactose (500–2500 mg/kg/day, maximum 50 g/day, divided in up to six doses/day) to the diet improves glycosylation significantly after a few weeks, although the transferrin glycoform pattern does not fully normalize. This treatment improves liver transaminases and antithrombin levels and in some patients the hormonal status. The effect of D-galactose on hypoglycemic episodes, cardiomyopathy, and myopathy is not yet clear.

Disorders of Golgi Homeostasis: TMEM199-CDG, CCDC115-CDG, ATP6AP2-CDG, and ATP6AP1-CDG

Clinical Manifestations

These four disorders are clinically and biochemically indistinguishable. They have been described with liver function anomalies, cholestasis, fibrosis, and cirrhosis with liver failure, necessitating liver transplantation in a few patients. The phenotype resembles **Wilson disease**, especially because of low serum ceruloplasmin and copper levels, but there is no Kayser-Fleischer ring. In CCDC115-CDG there are frequently also neurologic features. The intellectual outcome is variable. Additional abnormalities include hypercholesterolemia

and elevated alkaline phosphatase. In ATP6AP1-CDG there is also immunologic involvement.

Pathophysiology
TMEM199-, CCDC115-, ATP6AP1-CDG, and ATP6AP2-CDG are important for Golgi homeostasis. The exact pathologic mechanism is not yet known, but it is hypothesized that the secondary Golgi dysfunction affects and delays the normal glycosylation process.

Diagnosis
The primary screening method in a patient with one of these suspected CDGs is serum transferrin glycoform analysis by mass spectroscopy or TIEF analysis. Patients show a type 2 pattern (see Fig. 107.9). ApoC-III TIEF is abnormal. Glycomics results by MALDI-TOF analysis are characteristic but cannot discriminate between the three defects. The final diagnosis requires pathogenic variant analysis. TMEM199-CDG and CCDC115-CDG are autosomal recessive, whereas ATP6AP1-CDG and ATP6AP2-CDG are X-linked.

Treatment
Treatment is supportive. Two patients successfully underwent liver transplantation.

Manganese Transporter Defect: SLC39A8-CDG
Clinical Manifestations
This intriguing disorder was originally described as a neurologic disease with hypotonia, seizures (hypsarrhythmia), and developmental disability. Some of the later-described patients had severe skeletal dysplasia with rhizomelic chondrodysplasia, craniosynostosis, and dwarfism. Mitochondrial dysfunction (Leigh disease, cerebral lactic acidemia, dystonia) may also be present.

Pathophysiology
SLC39A8 is a membrane transporter, responsible for manganese (Mn) transmembrane transport. SLC39A8 deficiency affects all Mn-dependent enzymes and therefore different parts of the metabolism. Because several glycosyltransferases (e.g., β-1,4-galactosyltransferase) are Mn dependent, a secondary Golgi glycosylation occurs with a type 2 glycosylation defect. Low serum Mn levels are suggestive but not always present in patients.

Diagnosis
The primary screening method in a suspected patient with SLC39A8-CDG is **serum transferrin glycoform analysis** by mass spectroscopy or TIEF analysis. Patients show a type 2 pattern (see Fig. 107.9). MALDI-TOF analysis is suggestive but not discriminative. Low serum Mn levels are not always present in patients. The final diagnosis requires pathogenic variant analysis. SLC39A8-CDG is an autosomal recessive disease. The exact incidence for this rare disease is unknown.

Treatment
Besides supportive treatment, a few patients have shown biochemical and clinical improvement (better seizure control) with oral D-galactose (1-3.75 g/kg/day) and manganese (II)-sulfate monohydrate (15-20 mg/kg/day) therapy (see Table 107.7).

CONGENITAL DISORDERS OF DEGLYCOSYLATION
N-Glycanase 1 Deficiency (*NGLY1* Defect)
Clinical Manifestations
Patients with NGLY1 deficiency have a glycosylation disorder, but not from the deficient synthesis; rather, it is caused by deficient breakdown of glycoproteins. The phenotype comprises severe CNS involvement, microcephaly, intellectual disability, seizures, neuropathy, movement disorders, and hypotonia. The presence of *alacrimia* or hypolacrimia is highly suggestive for the diagnosis, but not all patients have problems with tearing. Other features include failure to thrive, IUGR, and liver involvement. Some patients have a recognizable oval face with a short nose, flat profile, and hypertelorism. Masklike face also occurs, imitating the phenotype of mitochondrial disorders, especially when serum lactic acid levels are also elevated.

Pathophysiology
N-glycanase is responsible for the deglycosylation of misfolded *N*-linked glycoproteins. The enzyme is essential for cutting off the glycans before the proteins are degraded in the ER. The exact disease pathomechanism, however, is not yet clear. Serum transaminase and α-fetoprotein levels are also frequently increased.

Diagnosis
Serum transferrin isoform analysis shows a normal pattern. In some patients the excretion of a specific urine biomarker (aspartylglucosamine; Neu5Ac1Hex1GlcNAc1-Asn) is present and can be used as screening. The final diagnosis requires genetic analysis.

NGLY1-CDG is an autosomal recessive disease. The most common pathogenic variant is c.1201A>T/p.R401X. The exact incidence of the condition is unknown, but >50 patients have been reported in the few years since the discovery of the disease.

Treatment
Only supportive treatment is available for the patient with NGLY1 deficiency.

THERAPEUTIC SUMMARY (SEE TABLE 107.7)
Most CDGs are treatable only with supportive therapy. The initially discovered oral mannose treatment in MPI-CDG (1 g/kg/day) has proved to be efficient for coagulation problems and protein-losing enteropathy but cannot prevent liver fibrosis in all patients. Liver transplantation in MPI-CDG has been successful in a few patients. Oral D-galactose in PGM1-CDG (1g/kg/day) can improve serum transaminases and coagulation and have a positive effect on endocrine function but cannot restore glycosylation fully. Seizure frequency improved in patients with SLC39A8-CDG receiving oral D-galactose treatment (1 g/kg/day) and oral manganese intake. The congenital myasthenic syndrome in DPAGT1-CDG, GFPT1-CDG, and GMPPB-CDG has been successfully treated with a high dose of cholinesterase inhibitors. Several CDGs have been positively controlled by transplantation, including DOLK-CDG (DK1-CDG; heart transplantation), PGM3-CDG (hematopoietic stem cell transplantation), ATP6AP1-CDG, and CCDC155-CDG (liver transplantation).

Patients with CAD-CDG show significant clinical improvement on receiving oral uridine therapy, especially with seizure control. Two children with SLC35C1-CDG–defective immune function improved on oral fucose therapy. GNE-CDG patients showed significant improvement in muscle strength on *N*-acetylmannosamine therapy.

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Chapter 108

Mitochondrial Disease Diagnosis

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See also Chapters 107.4, 638.2.

Mitochondrial diseases are multisystemic energy failure states with extensive clinical and genetic heterogeneity. Their common basis is best understood through recognition that mitochondria function as biologic "fuel cells" or "batteries," producing chemical energy in the form of adenosine triphosphate (ATP) by aerobic metabolism of nutrient-derived reducing equivalents, through the integrated function of the five-complex mitochondrial respiratory chain (RC) (Fig. 108.1). Mitochondria also play other essential roles that can be variably disrupted in disease states, such as regulating calcium homeostasis, diverse aspects of intermediary nutrient metabolism, nucleotide metabolism, and oxidative stress. Primary mitochondrial disease results from deficient RC function, which can be caused by pathogenic variants in genes that encode RC subunits, assembly factors or cofactors, components of mitochondrial DNA (mtDNA) metabolism and maintenance, or a host of other basic metabolic processes ongoing within mitochondria (Table 108.1). Approximately 1,500 proteins exist within the mitochondrial proteome of different tissues, with variants in more than 350 unique genes across both the nuclear and the mitochondrial genomes already implicated as causal in human mitochondrial disease.

Collectively recognized as the most common group of inherited metabolic diseases, **primary** (genetic based) mitochondrial disease has a combined minimal prevalence of 1 in 4,300 individuals across all ages. In addition, **secondary** mitochondrial dysfunction is broadly implicated in the pathogenesis of a host of complex diseases, ranging from metabolic syndrome to ischemia-reperfusion injury after stroke, to neurodegenerative diseases. Failure of high-energy-demand organs in mitochondrial diseases may clinically present as severe neurodevelopmental, cardiac, myopathic, renal, hepatic, endocrine, immune, gastrointestinal (GI), hearing, and vision disabilities, as well as global metabolic instability with lactic acidosis (Fig. 108.2) (see Tables 107.2 and 107.3). In most mitochondrial disorders, the phenotype may vary depending on the patient's age, the specific gene or genetic variant, or tissue affected. Particularly common mitochondrial disease clinical syndromes that present in children

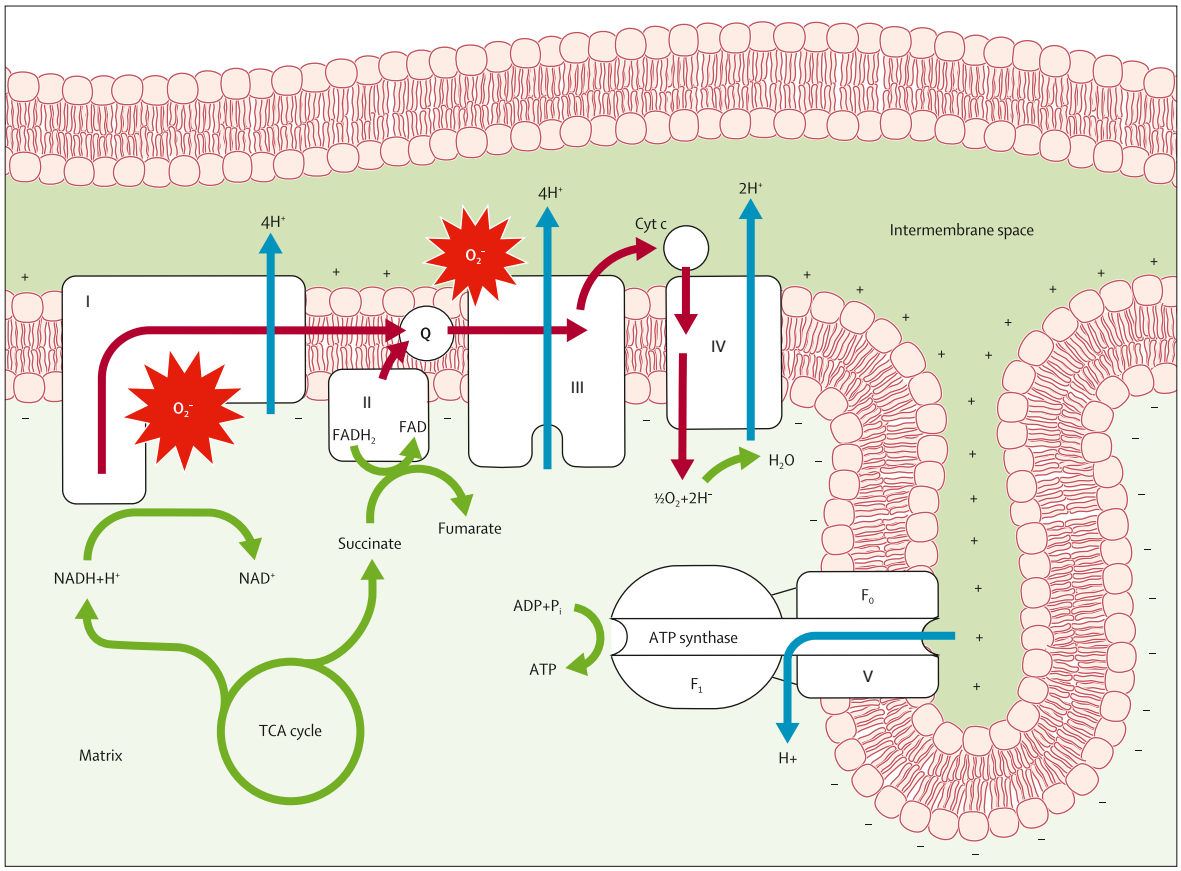

Fig. 108.1 Electron transport chain. The electron transport chain consists of four protein complexes (I-IV) coupled to a fifth (V) unlinked complex, ATP synthase. Together, these five complexes are known as the respiratory chain and are the site where oxidative phosphorylation (OXPHOS) occurs to generate energy. The transport chain accepts electrons from NADH (complex I) or $FADH_2$ (complex II) that have been produced by glycolysis, the formation of acetyl–coenzyme A, and the TCA cycle *(green arrows)*. Electrons flow from one complex to another *(red arrows)* because of the redox potential of each complex and lose a small amount of energy as they move through the chain. Three of the four complexes act as pumps, driven by electron flow, moving H^+ ions from the matrix to the intermembrane space *(blue arrows)*. This pumping builds a concentration gradient and creates an electrochemical force that is used by ATP synthase to produce ATP. Under normal conditions, this machinery provides almost all (90%) of the ATP in a cell. However, a small proportion of electrons escape the electron transport chain even under normal conditions and can react with oxygen and complexes I and III to form superoxide (O_2^-). ADP, Adenosine diphosphate; ATP, adenosine triphosphate; Cyt c, cytochrome *c*; Q, coenzyme Q; NADH, nicotinamide dinucleotide; Pi, inorganic phosphate; TCA, tricarboxylic acid cycle; FADH2, 1,5-dihydro-flavin adenine dinucleotide. (*Adapted from Hagberg H, Mallard C, Rousset CI, Thornton C. Mitochondria: hub of injury responses in the developing brain. Lancet Neurol. 2014;13[2]:217–232.*)

Table 108.1 Current Mitochondrial Gene Defects and Pathomechanisms

	GENE(S)
OXIDATIVE PHOSPHORYLATION DEFICIENCY	
Complex I subunits and assembly factors	*NDUFA1, NDUFA2, NDUFA6, NDUFA9, NDUFA10, NDUFA11, NDUFA12, NDUFA13 NDUFB3, NDUFB8, NDUFB9, NDUFB10, NDUFB11, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8, NDUFV1, NDUFV2, NDUFAF1, NDUFAF2, NDUFAF3, NDUFAF4, NDUFAF5, NDUFAF6, NDUFAF7, NDUFAF8, ACAD9, ECSIT, FOXRED1, NUBPL, TIMMDC1, TMEM126B, MT-ND1, MT-ND2, MT-ND3, MT-ND4, MT-ND4L, MT-ND5, MT-ND6*
Complex II subunits and assembly factors	*SDHA, SDHB, SDHC, SDHD, SDHAF1, SDHAF2*
Complex III subunits and assembly factors	*UQCRB, UQCRC2, UQCRFS1, UQCRQ, CYC1, BCS1L, HCCS, TTC19, LYRM7, UQCC2, UQCC3, MT-CYB*
Complex IV subunits and assembly factors	*COX4I1, COX4I2, COX5A, COX6A1, COX6B1, COX7B, COX8A, NDUFA4, SURF1, SCO1, SCO2, COX10, COX15, COA3, COA5, COA6, COA7, COX14, COX20, FASTKD2, PET100, PET117, CEP89, MT-CO1, MT-CO2, MT-CO3*
Complex V subunits and assembly factors	*ATP5A1, ATP5D, ATP5E, ATPAF2, TMEM70, USMG5, MT-ATP6, MT-ATP8*
DISORDERS OF MITOCHONDRIAL DNA MAINTENANCE	
Nucleotide pool maintenance	*ABAT, AK2, DGUOK, RRM2B, SAMHD1, SUCLA2, SUCLG1, TK2, TYMP*
Replication, maintenance, and transcription of mtDNA	*DNA2, FBXL4, MGME1, MPV17, POLG, POLG2, SSBP1, SLC25A4, TWNK*
MITOCHONDRIAL TRANSLATION DEFECTS	
Mitochondrial tRNAs	*MT-TA, MT-TC, MT-TD, MT-TD, MT-TE, MT-TF, MT-TG, MT-TH, MT-TI, MT-TK, MT-TL1, MT-TL2, MT-TM, MT-TN, MT-TP, MT-TQ, MT-TR, MT-TS, MT-TT, MT-TV, MT-TW, MT-TY*
Mitochondrial aminoacyl-tRNA synthetases	*AARS2, CARS2, DARS, DARS2, EARS2, FARS2, GARS, HARS2, IARS, IARS2, KARS, LARS, LARS2, MARS2, NARS2, PARS2, QARS, RARS2, SARS2, TARS2, VARS2, WARS2, YARS2*
tRNA modification	*ELAC2, MTFMT, NSUN3, PDE12, QRSL1, TRIT1, TRMT5, TRMT10C, TRNT1*
Mitochondrial rRNA	*MT-RNR1, MT-RNR2*
RNA processing	*PNPT1*
Mitoribosome subunits and assembly	*ERAL1, MRPL3, MRPL12, MRPL44, MRPS2, MRPS7, MRPS16, MRPS22, MRPS23, MRPS34, MRM2, RMND1*
Protein synthesis	*C12orf65, GFM1, GFM2, GTPBP3, GUF1, LRPPRC, MTO1, MTPAP, PUS1, TACO1, TRMU, TSFM, TUFM*
MITOCHONDRIAL QUALITY CONTROL DEFECTS	
Mitochondrial membrane phospholipid and import machinery	*AGK, CHKB, DNAJC19, GFER, MIPEP, PAM16, PLA2G6, PMPCA, SERAC1, SLC25A3, SLC25A10, SLC25A12, SLC25A22, TAZ, TIMM8A, TIMM50, XPNPEP3*
Mitochondrial dynamics	*DNM1L, GDAP1, MFF, MFN2, MSTO1, OPA1, STAT2, TRAK1, YME1L1*
MICOS complex	*CHCHD10, QIL1, SLC25A46*
ER-mitochondrial tethering	*EMC1*
Mitochondrial protein quality control	*AFG3L2, ATAD3A, CLPB, CLPP, CLPX, HSPA9, HSPD1, HSPE1, LONP1, PITRM1, SACS, SPG7, TRAP1*
Toxicity	*ECHS1, ETHE1, HIBCH*
Antioxidant defense	*NNT, TXN2*
METABOLIC DEFECTS	
Tricarboxylic acid cycle enzymes	*ACO2, DHTKD1, FH, IDH3A, IDH3B, MDH2, OGDH*
Pyruvate metabolism	*DLAT, DLD, MPC1, PC, PDHA1, PDHB, PDHX, PDK3, PDP1, PDPR*
Fatty acid metabolism	*CRAT, ETFA, ETFB, ETFDH, FA2H, HSD17B10, PYCR1, SLC25A1*
CoA metabolism and transport	*COASY, PANK2, SLC25A42*
VITAMIN AND COFACTOR METABOLISM DEFECTS	
Coenzyme Q_{10} biosynthesis	*COQ2, COQ4, COQ5, COQ6, COQ7, COQ8A, COQ8B, COQ9, PDSS1, PDSS2*
Iron-sulfur cluster protein biosynthesis	*ABCB7, FDXR, FDX1L, FXN, ISCA1, ISCA2, ISCU, LYRM4, NFS1, NFU1*
Lipoic acid biosynthesis	*BOLA3, GLRX5, IBA57, LIAS, LIPT1, LIPT2, MECR*
Cytochrome *c*	*CYCS*
Biotin metabolism	*BTD, HLCS*
Thiamine metabolism and transport	*SLC19A2, SLC19A3, SLC25A19, TPK1*
Mitochondrial one-carbon metabolism	*SLC25A26, SLC25A32*
Heavy metal metabolism	*SLC25A24, SLC33A1, SLC39A8*
Selenoprotein biosynthesis	*SECISBP2, SEPSECS*
NADPH metabolism	*NADK2, NAXD, NAXE*
Riboflavin metabolism and transport	*FLAD1, SLC52A2, SLC52A3*
OTHER CELLULAR DEFECTS ASSOCIATED WITH MITOCHONDRIAL DYSFUNCTION	
Ca^{2+} homeostasis	*ANO10, C19ORF70, CISD2, CYP24A1, MICU1, MICU2, WFS1*
Heme biosynthesis	*ABCB6, ALAS2, SFXN4, SLC25A38*
Apoptosis defects	*AIFM1, APOPT1, DIABLO, HTRA2, PTRH2*
DNA repair	*APTX, XRCC4*
Miscellaneous or unknown function	*ALDH1B1, ALDH18A1, BDH1, CA5A, CTBP1, C1QBP, C19ORF12, DCC, DIAPH1, FHF1, KIF5A, OPA3, PNPLA4, PNPLA8, POP1, PPA2, ROBO3, RTN4IP1, SLC44A1, STXBP1, TANGO2, TMEM65, TMEM126A*

mtDNA, Mitochondrial DNA; MICOS, mitochondrial contact site and cristae organizing system; ER, endoplasmic reticulum.
From Rahman J, Rahman S: Mitochondrial medicine in the omics era. *Lancet.* 2018;391:2560–2574, Table 1.

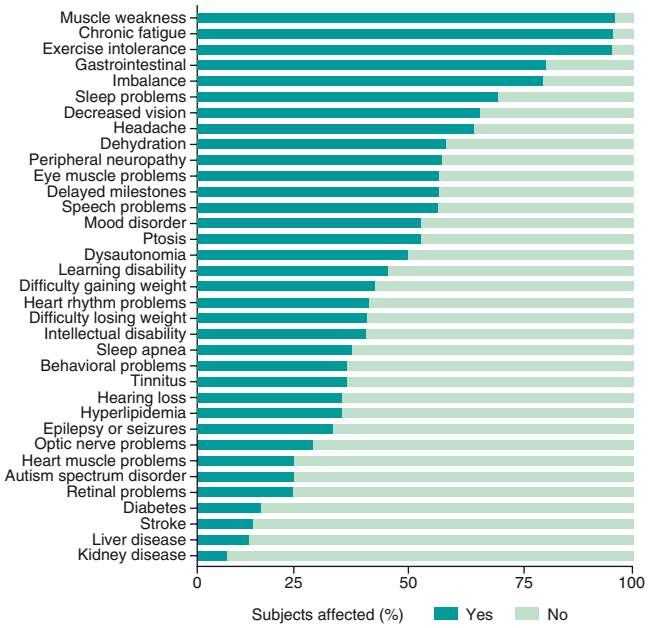

Fig. 108.2 Mitochondrial disease subject cohort experienced symptoms. Frequency of experienced symptoms as reported by the Rare Diseases Clinical Research Network (RDCRN) self-reported cohort revealed muscle weakness, chronic fatigue, exercise intolerance, imbalance, and gastrointestinal problems to be the top five common symptoms. (*Modified from Zolkipli-Cunningham Z, Xiao R, Stoddart A, et al. Mitochondrial disease patient motivations and barriers to participate in clinical trials. PLoS ONE. 2018;13[5]:e0197513. Fig. 2.*)

include **Leigh syndrome** (for which there are more than 110 causal genes; for gene listing visit https://search.clinicalgenome.org/kb/conditions/MONDO:0009723.), **mtDNA depletion syndrome** (MDS, for which there several dozen causal genes), **mtDNA deletion syndromes (Pearson**, **Kearns Sayre)**, primary lactic acidosis, and pyruvate dehydrogenase deficiency. Common clinical features in children present in at least 90% of patients include fatigue, exercise intolerance, weakness, GI and hepatic dysfunction, ataxia, and developmental delay. Thus mitochondrial diseases present to and must be considered by clinicians across every medical specialty.

Reversible infantile respiratory chain deficiency is an unusual mitochondrial disorder that presents in early (≤3 months) infancy with muscle weakness, hypotonia, poor feeding, and lactic acidosis and spontaneously resolves beginning around 1 year of age. Responsible maternal inherited genes include *mt-tRNAGlu (MT-TE)* (in 100%) and possible modifier genes *(EARS2, TRMU)*. Muscle biopsy may show ragged red fibers.

Patients with suspected mitochondrial disease often have an extensive phenotypic heterogeneity without a common biomarker, which presents a challenge to the accurate **clinical diagnosis** of mitochondrial disorders. Lactate and growth differentiation factor 15 (GDF-15) are screening tests that may be elevated in *some* mitochondrial diseases, particularly those involving mtDNA deletions or depletion. In addition, their extensive genetic heterogeneity involving known etiologies in >300 nuclear genes and all 37 mtDNA genes can make the accurate **genetic diagnosis** of an individual patient challenging. The diagnostic uncertainty can be further compounded by poor genotype-phenotype correlations and variable clinical presentations of individual gene disorders, high locus heterogeneity (i.e., multiple different causal disease genes) for similar clinical phenotypes, incomplete penetrance for some gene disorders, variable life stressors or environmental exposures that may exacerbate a given child's disease, and the unique biologic aspects of maternal inheritance for the subset of mitochondrial diseases caused by mtDNA pathogenic variants.

WHEN TO SUSPECT MITOCHONDRIAL DISEASE

Because of failure in the ability to generate cellular energy, mitochondrial diseases can involve any organ system at any age (see Fig. 108.2). Mitochondrial disease should be suspected when classic symptoms are present or if unexplained symptoms occur in three or more apparently unrelated organs. Individuals may present with a vast array of symptoms, including fatigue, muscle weakness, exercise intolerance, metabolic strokes, seizures, cardiomyopathy, arrhythmias, developmental or cognitive disabilities, autism, diabetes mellitus, and other endocrinopathies (adrenal, thyroid), dysautonomia, and autoimmune disorders, as well as impairment of hearing, vision, growth, liver, GI, or kidney function. Although individuals may have just one or a few symptoms and a fluctuating disease course in terms of symptom severity, most patients with primary mitochondrial disease tend to develop *progressive* symptoms over time. A study of patients with mitochondrial diseases showed an average of 16 different clinically significant symptoms per patient, with a range of 7-35. When considering the diagnosis, it is helpful to recognize that most symptoms of mitochondrial disease involve *functional,* rather than structural, problems.

When mitochondrial disease is considered in the differential diagnosis, it is often helpful to obtain several **laboratory screening** studies for common biochemical features of mitochondrial disease and overlapping disorders at baseline and, if unrevealing, during an acute illness or period of decompensation. Blood-based metabolic screening studies include comprehensive chemistry panel, complete blood count with differential, blood lactate and pyruvate, plasma amino acid quantitative analysis, carnitine analysis (total, free, acyl-carnitine profile), ammonia, creatine kinase, and testing for common secondary manifestations of mitochondrial disease (e.g., thyroid screen, lipoprotein profile, hemoglobin A_{1c}). Urine-based metabolic screening studies include urinalysis, urine organic acid quantitative analysis, and urine amino acid quantitative analysis. Consideration should also be given for screening for congenital disorders of glycosylation or vitamin deficiencies, which may have overlapping clinical features in some cases with mitochondrial disease. Lactic acidemia is neither highly sensitive nor specific for primary mitochondrial disease, but laboratory findings suggestive of primary mitochondrial disease include elevations of blood lactate, pyruvate, lactate:pyruvate ratio, alanine, ratios of alanine to lysine (>3) and alanine to the sum of phenylalanine and tyrosine (>4), and anion gap. Biochemical alterations further suggestive of mitochondrial disease may include secondary impairment of fatty acid oxidation with elevation of dicarboxylic acids on acyl-carnitine profile, increased branched-chain amino acids and proline on plasma amino acid analysis, increased tricarboxylic acid cycle intermediates and lactate excretion on urine organic acid analysis, and generalized aminoaciduria on urine amino acid analysis. **GDF-15** may be a useful screening test for mitochondrial depletion-based myopathies.

Similarly, when mitochondrial disease is considered in the differential diagnosis, obtaining additional **clinical evaluations** to carefully phenotype the patient for prevalent or highly morbid and potentially modifiable features of mitochondrial disease is important. Because many individuals with mitochondrial disease develop problems with their *vision* (reduced visual acuity not correctable with glasses, photophobia or nyctalopia with reduced peripheral vision associated with retinal disease or optic atrophy, ophthalmoplegia, ptosis), *hearing* (high-frequency sensorineural hearing loss), and *heart* (arrhythmia, conduction block, cardiomyopathy), carefully evaluating for involvement of these high-energy systems is indicated. **Neurologic** evaluation is essential because many mitochondrial disease patients experience a range of *central* (metabolic stroke in cortical or deep gray matter, including basal ganglia, midbrain, and/or brainstem; white matter changes; seizures; ataxia; movement disorder; migraine; cognitive changes), *peripheral* (axonal sensorimotor neuropathy), or *autonomic* nervous system dysfunction; brain imaging (MRI), spectroscopy (MRS), and, on occasion, electromyogram or nerve conduction velocity (EMG/NCV) studies can be helpful to support the diagnosis. Formal **exercise physiology** evaluation can also be useful to quantify and advise patients on their exercise capacity and safety, with some specific features (e.g., reduced Vo_2 maximal capacity) suggestive of quantifiable mitochondrial dysfunction. A **sleep** study may be useful for individuals with sleep dysfunction because sleep disorders may mimic mitochondrial disease symptoms, and sleep problems are common and potentially treatable in mitochondrial disease. **Gastrointestinal** symptoms are common and underrecognized in mitochondrial disease patients, usually involving dysmotility of any portion of the GI tract with reflux, swallowing dysfunction, delayed gastric emptying,

feeding and/or growth problems, pseudoobstruction, malabsorption, and constipation. **Endocrine** abnormalities are also common but underappreciated in many patients, including pituitary, adrenal, thyroid, and pancreatic dysfunction.

Such careful phenotyping of patients with suspected mitochondrial disease can thus provide reassurance that the common, and potentially treatable, clinical aspects of mitochondrial disease are not present although they may develop over time, or conversely if identified, increase diagnostic suspicion and direct further diagnostic evaluation. A screening tool for mitochondrial diseases is noted in Table 108.2. The differential diagnosis of mitochondrial disorders is extensive (Table 108.3).

MITOCHONDRIAL DISEASE INHERITANCE

Primary mitochondrial disease may result from variants in either nuclear genes or mtDNA genes, which may be inherited from a parent or occur de novo in an affected individual (for a list of genes curated for their association with primary mitochondrial disease, visit https://search.clinicalgenome.org/kb/affiliate/10027). Thus all *mendelian* (autosomal recessive, autosomal dominant, X-linked) or *maternal* (mtDNA) inheritance patterns can be consistent with mitochondrial diseases. Obtaining a detailed, three-generation pedigree is important to potentially highlight the specific inheritance pattern in a family. Individuals with inherited mtDNA disorders may report family members related through their **maternal** lineage (both males and females may be affected, but only affected individuals will be connected through the female germline), with a range of functional problems in different organs, such as migraines, fatigue, exercise intolerance, stroke, diabetes mellitus, thyroid dysfunction, irritable bowel syndrome, mood disorder, or vision and hearing problems (for a list of curated mtDNA variants, visit https://erepo.clinicalgenome.org). Inherited **X-linked** disorders typically present with symptoms only or, more severely, in males related through unaffected or minimally affected females. **Autosomal recessive** disorders are common in pediatric mitochondrial disease, particularly in consanguineous pedigrees, where a rare variant in the general population becomes enriched and passed down through both maternal and paternal lineages to become homozygous in the affected proband and also affect multiple individuals in a given generation without having affected individuals in earlier generations. **Autosomal dominant** variants may occur de novo or are passed on from either parent to their child, although many disorders may have reduced penetrance, which may make the genetic disorder appear to skip a generation. Identifying a likely inheritance pattern through pedigree analysis can inform accurate interpretation of large-scale genetic diagnostic evaluations, such as multigene sequencing and deletion/duplication analysis panels and exome or genome sequencing. Establishing a correct genetic diagnosis for mitochondrial disease in an affected individual is essential to enable reliable recurrence risk counseling and testing options in a given family, whether in a future pregnancy by chorionic villus sampling (CVS, typically performed at 10-12 weeks' gestation) or amniocentesis (typically performed at 16-20 weeks' gestation) or in the in vitro fertilization (IVF) setting with preimplantation genetic testing (PGT) for a specific disease-causing variant. Of note, PGT is readily available to families for known nuclear gene disorders but remains of limited availability in known pathogenic mtDNA variants.

Special mention is warranted to consider the unique aspects of *maternal inheritance* that typify mtDNA disorders. More than 300 disease-causing mtDNA variants have been identified, with extensive variation in disease manifestations and features. Most disease-causing variants are present in only a portion of an individual's mtDNA genomes, a concept known as **heteroplasmy**. For heteroplasmic mtDNA variants, the precise pathogenic variant level (percent) can vary between an individual's different tissues and can change over time, with symptom severity corresponding to different threshold pathogenic variant levels that can be difficult to define and that typically vary between organs. An individual's mtDNA genome background set of fixed sequence variants, known as a **haplogroup**, can also influence the penetrance or severity of an mtDNA disease. When a novel or rare mtDNA variant is identified in a given individual, it may be helpful to use highly sensitive sequencing methods to test the levels of that pathogenic variant (which may be accurate to detect 1% of pathogenic variant levels) in their different tissues (blood, urine, buccal, skin cells, muscle), as well as tissues from their mother or maternal relatives, to accurately determine whether it may be causal of disease in that family. Research-based functional testing may also be necessary to characterize fully the effects of a newly recognized mtDNA variant. When it is not known whether an mtDNA variant is maternally inherited or occurs de novo, the recurrence risk to future offspring of their asymptomatic parent is empirically estimated at 1 in 25 (4%), although the empirical recurrence risk rises to 1 in 2 (50%) when the mother is symptomatic.

Table 108.2 Mitochondrial Disease Criteria

	FEATURES	
Muscular	Myopathy Abnormal EMG Motor developmental delay Exercise intolerance	Maximal score for muscle is 2
Neurologic	Developmental delay or ID Speech delay Dystonia Ataxia Spasticity Neuropathy Seizures or encephalopathy	Maximal score for neurologic is 2
Multisystem	Any gastrointestinal tract disease Growth delay or failure to thrive Endocrine Immune Eye (vision) or hearing Renal tubular acidosis Cardiomyopathy	Maximal score for multisystem is 3
Total clinical		**Total maximal clinical score is 4**
Metabolic	Lactate high at least 2×: (score 2) Alanine high at least 2× Krebs cycle intermediates[a] Ethyl malonic and methyl malonic acid 3 methyl glutaconic acid CSF lactate, alanine	
Imaging/other	Leigh disease (score 2) Strokelike episodes (score 2) Lactate peak on MRS Leukoencephalopathy with brainstem and spinal cord involvement[b] Cavitating leukoencephalopathy[b] Leukoencephalopathy with thalamus involvement[b] Deep cerebral white matter involvement and corpus callosum agenesis[b]	Total metabolic and MRI maximal score is 4
Total MDC score (clinical, metabolic, imaging)		**Total maximal score is 8**

[a]Krebs cycle intermediates: alpha-ketoglutarate, succinate, fumarate.

[b]Numerous MRI patterns characteristic of mitochondrial disease have been described in addition to Leigh syndrome and basal ganglia with brainstem involvement. We include leukoencephalopathy with brainstem and spinal cord involvement *(DARS2)*, cavitating leukoencephalopathy *(LYRM7)*, leukoencephalopathy with thalamus involvement *(EARS2)*, and deep cerebral white matter involvement and corpus callosum agenesis *(NUBPL)*.

Every element scores 1 unless indicated differently. The severity of each finding is not taken into account because of the progressive nature of the disease. A total score of 1 indicates unlikely mitochondrial disorder; score 2–4, possible mitochondrial disorder; score 5–7, probable mitochondrial disorder; and score ≥8, definite mitochondrial disorder.

COX, Cytochrome c oxidase; EMG, electromyography; L/P, lactate/pyruvate; SDH, succinate dehydrogenase.

From Witters P, Saada A, Honzik T, et al. Revisiting mitochondrial diagnostic criteria in the new era of genomics. *Genetics Med.* 2018;20(4):444–451, Table 2.

Table 108.3 Differential Diagnosis of Selected Phenotypes Commonly Associated with Mitochondrial Disease

PHENOTYPE	MITOCHONDRIAL CAUSE	LIMITED DIFFERENTIAL DIAGNOSIS
Dystonia	Leigh syndrome, deafness-dystonia syndrome, other mitochondrial encephalomyopathies	Biotinidase deficiency, thiamine transporter deficiency 2, *ADAR* pathogenic variants (Aicardi-Goutières syndrome 6), organic acidemias (especially glutaric aciduria type I), NBIA, acute (viral) necrotizing encephalopathy, pathogenic variants in *NUP62, RANBP2,* and *PDE8B*, primary genetic dystonias
Epileptic encephalopathy	Alpers-Huttenlocher syndrome, many other mitochondrial disorders	Many genetic epileptic encephalopathies, including Dravet syndrome and *KCNQ2* pathogenic variants, pyridoxine-dependent epilepsies (antiquitin deficiency, PNPO deficiency), viral encephalitis
Progressive myoclonic epilepsy	MERRF	Ramsay Hunt syndrome, Unverricht-Lundborg disease, Lafora body disease, sialidosis, *PRICKLE1* pathogenic variants
Leukoencephalopathy	Complex I deficiency, complex II deficiency, SURF1 deficiency (rarely), disorders of mitochondrial translation and Fe-S cluster assembly	Vanishing white matter disease, lysosomal storage disorders, Canavan disease, Alexander disease, Pelizaeus-Merzbacher(-like), hypo/dysmyelination
Ataxia	*ADCK3* pathogenic variants, ataxia-neuropathy syndromes, for example, SCAE, MIRAS, MERRF, NARP, disorders of coenzyme Q_{10} biosynthesis	Spinocerebellar ataxias, CAPOS syndrome
Demyelination	MNGIE	ADEM, multiple sclerosis
Peripheral neuropathy	Pathogenic variants in *POLG, MPV17, KARS,* and *SURF1*; part of multisystem disease in many mitochondrial disorders, for example, MNGIE	Other nonmitochondrial genetic causes of Charcot-Marie-Tooth syndromes, riboflavin transporter deficiency, toxic neuropathies, critical illness
Ptosis and ophthalmoplegia	PEO, KSS, MNGIE, MELAS	Some congenital myopathies, pseudo-upgaze impairment in *OPMD,* horizontal gaze palsy and scoliosis (*ROBO3* pathogenic variant)
Optic neuropathy	LHON, ADOA, Leigh syndrome	Toxic optic neuropathy (e.g., methanol, cyanide, tobacco)
Hypertrophic cardiomyopathy with lactic acidosis	Complex I deficiency, *TMEM70* pathogenic variants, Sengers syndrome (AGK deficiency), disorders of mitochondrial translation	Viral infection
Dilated cardiomyopathy with lactic acidosis	Barth syndrome, disorders of mitochondrial phospholipid remodeling, other mitochondrial cardiomyopathies	Viral infection
Exocrine pancreatic insufficiency	Pearson syndrome	Cystic fibrosis
Diabetes and deafness	MIDD, other mtDNA pathogenic variants	Type 2 diabetes mellitus with incidental nonsyndromic deafness
Sideroblastic anemia	Pearson syndrome, MLASA, TRNT1 deficiency, *PUS1* or *YARS2* pathogenic variants	Blackfan-Diamond syndrome, Schwachman-Diamond syndrome, X-linked sideroblastic anemia
B-cell immune deficiency	TRNT1 deficiency	Primary immunodeficiency disorder
Liver failure	Mitochondrial DNA (mtDNA) depletion syndromes	NBAS, LARS, and IARS deficiencies, viral infection, lysosomal storage disorders, other syndromic genetic conditions
Renal tubulopathy/failure	Pearson and Kearns-Sayre syndromes, *RMND1*-related disease	Gitelman syndrome, Fanconi Bickel (*SLC2A2* pathogenic variants) syndrome, other syndromic genetic conditions
Myopathy	Part of multisystem disease in many mitochondrial disorders, especially mtDNA depletion syndromes	Congenital muscular dystrophies, myositis, many other disorders
Rhabdomyolysis	Mitochondrial myopathies (e.g., *MTCO1, MTCO2, MTCO3,* and *MTCYB* pathogenic variants)	*LPIN1* pathogenic variants, fatty acid oxidation defects (VLCAD, LCHAD), TANGO deficiency, glycolytic defects, toxic, postexercise
Low copper	Cytochrome oxidase deficiency	Menkes, *SLC33A1* pathogenic variants
Complex multisystem disorders	Many mitochondrial disorders, particularly in childhood	Congenital disorders of glycosylation, peroxisomal disorders, lysosomal storage disorders, other syndromic genetic conditions

ADEM, Acute disseminated encephalomyelitis; ADOA, autosomal dominant optic atrophy; CAPOS, cerebellar ataxia, areflexia, pes cavus, optic atrophy and sensorineural hearing loss; Fe-S, iron-sulfur; KSS, Kearns-Sayre syndrome; LHON, Leber hereditary optic neuropathy; MERRF, myoclonic epilepsy with ragged red fibers; MIDD, maternally inherited diabetes and deafness; MIRAS, mitochondrial recessive ataxia syndrome; MLASA, myopathy, lactic acidosis, sideroblastic anemia; MNGIE, mitochondrial neurogastrointestinal encephalomyopathy; NBIA, neurodegeneration with brain iron accumulation; PEO, progressive external ophthalmoplegia; SCAE, spinocerebellar ataxia with epilepsy.
Modified from Parikh S, Karaa A, Goldstein A, et al. Diagnosis of "possible" mitochondrial disease: an existential crisis. *J Med Genet.* 2019;56(3):123–130, Table 1.

DIAGNOSTIC TESTING FOR MITOCHONDRIAL DISEASE

The diagnosis of mitochondrial disease relies foremost on genetic testing (genomic analysis), with biochemical screens useful in blood or urine and invasive tissue testing often seen as secondary or sometimes not required at all (Fig. 108.3).

When the clinical evaluation—medical history; detailed review of systems; careful physical, neurologic, and dysmorphic examinations; pedigree review; blood- and urine-based biochemical screening studies; and additional phenotyping clinical evaluations—is suggestive of mitochondrial disease, a range of clinical diagnostic testing options can be pursued. Without a known molecular etiology in an affected family member, first-line genetic diagnostic testing should include whole exome sequencing (WES) and mtDNA genome sequencing using methodologies that will detect both single-nucleotide variants and larger-scale gene deletions and duplications. WES is more comprehensive for genes known not only to cause mitochondrial disease but also to cause all human genetic diseases. The rationale for this diagnostic testing approach includes the following factors:

- The mtDNA genome sequence is often included at no extra cost when clinical WES is ordered in blood or buccal samples but may need to be repeated in a symptomatic tissue (e.g., muscle, liver) to detect heteroplasmic mtDNA variants that may not be present in more accessible tissues such as blood or buccal cells.
- The utility of performing concurrent proband and both parental sample sequencing *(trio-based testing)*, as usually pursued with WES but not panel-based testing, thereby allowing concurrent segregation analysis of a suspected pathogenic variants as well as ready identification of de novo dominant variants in the proband.
- The ability to use WES raw data (either on a research basis or for reanalysis at a later date by the clinical diagnostic laboratory) to highlight and/or identify "novel" gene disorders not previously recognized or associated with human disease.

Exome sequencing including mtDNA is estimated to identify the definitive genetic etiology for mitochondrial disease in at least 60% of patients in whom it is strongly suspected. Whole genome sequencing (WGS) is increasingly used when routine molecular testing such as WES with mtDNA sequencing is unrevealing of the genetic etiology. A mitochondrial disease community resource to centrally curate all mitochondrial disease, gene, and variant knowledge across both genomes is publicly accessible at https://mseqdr.org/.

Tissue-based diagnostic testing has decreased in frequency as a frontline test in all patients with suspected mitochondrial disease, although it still has clinical utility. These include (1) in the setting of rapidly deteriorating clinical status when genetic testing results may not be available in a timely fashion; (2) when a variant of uncertain significance identified on genomic testing has unclear biochemical consequences; and (3) when uninformative genomic sequencing in blood in an individual with myopathy or muscle symptoms raises concern for other disease processes that may be evident on histology, electron microscopy, immunohistochemistry, or enzymatic tissue testing. In addition, some mitochondrial diseases are only evident by tissue-based diagnostic testing. These include mtDNA deletion disorders (typically involving several thousand nucleotides) not present in blood that cause **chronic progressive external ophthalmoplegia** (CPEO) or **Kearns-Sayre syndrome** (KSS) spectrum disorder, as well as different tissue (muscle or liver)-specific mtDNA depletion disorders (e.g., reduced mtDNA tissue content) that confirm a mitochondrial pathophysiology in a given patient and highlight a likely underlying nuclear gene cause for their disease, because mtDNA maintenance requires a host of nuclear-encoded proteins. **Electron transport chain enzyme** activity analyses are the accepted gold standard to evaluate for mitochondrial dysfunction in a previously frozen tissue sample. Skin biopsies are useful to establish fibroblast cell lines in which these same studies of mitochondrial function can be clinically performed. If detected, abnormalities can be revealing of a specific type of mitochondrial disorder, although not all mitochondrial diseases may be expressed or detectable in skin analysis. Thus if fibroblast testing is unrevealing, more invasive tissue studies may subsequently need to be pursued. RNA sequencing may lead to identification of the genetic etiology in an additional 10% of individuals with mitochondrial disease in

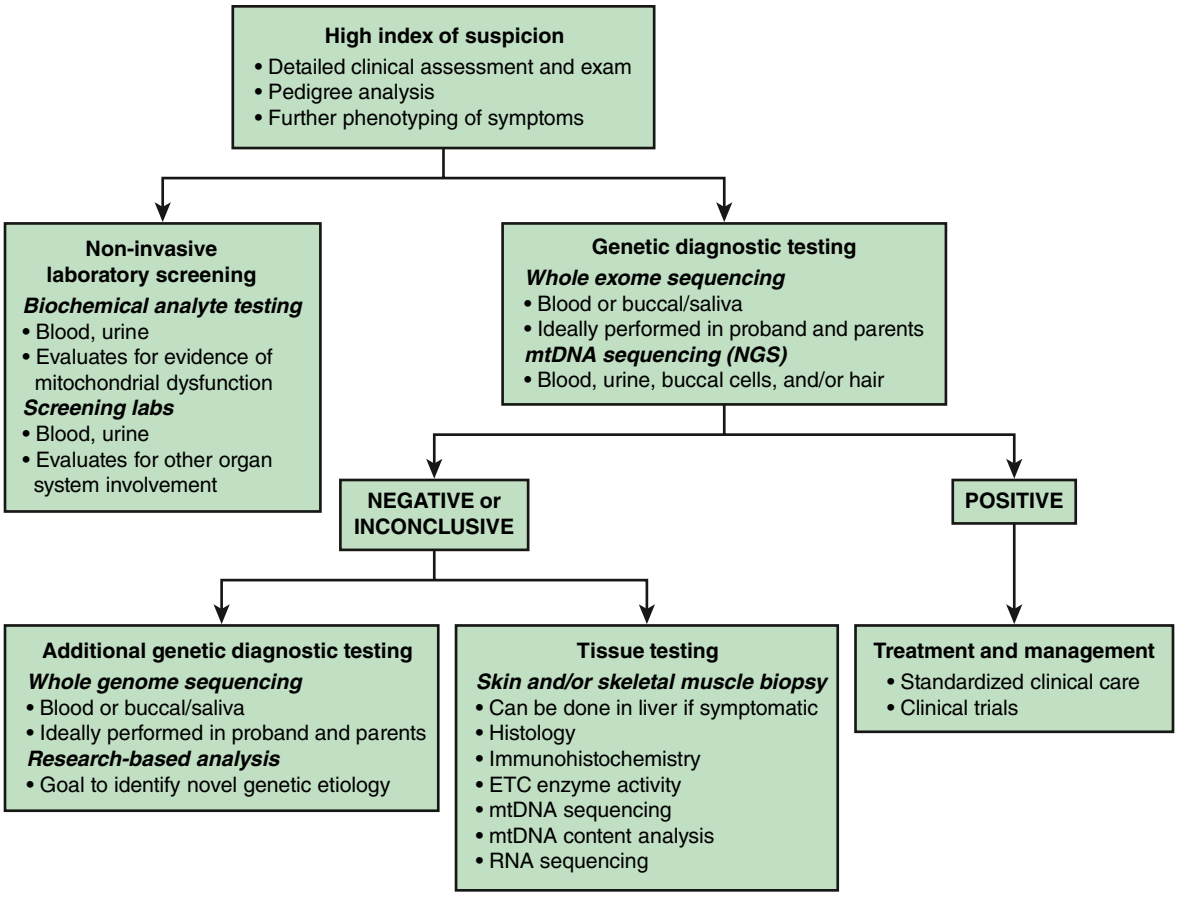

Fig. 108.3 Diagnostic algorithm for suspected mitochondrial disease. (*Modified from Murarescu CC, McCormick EM, Falk MJ. Mitochondrial disease: advances in clinical diagnosis, management, therapeutic development, and preventative strategies. Curr Genet Med Rep. 2018;6:62–72.*)

whom DNA-based sequencing did not identify the disease-causing variants. Only a small number of clinical laboratories offer this testing; this test can be performed in muscle, fibroblasts, or blood. Fibroblast cell lines, and occasionally blood-based lymphoblastoid cell lines, also provide a minimally invasive cell source to allow other clinical enzymatic analyses to be performed, as well as novel disease gene validation and research-based therapeutic modeling.

TREATMENT PRINCIPLES FOR MITOCHONDRIAL DISEASE

Effective therapies for both primary and secondary mitochondrial diseases are lacking. Clinical complexity and imprecisely defined or understood biochemical phenotypes of different mitochondrial disease subtypes have made it difficult for clinicians to effectively apply or monitor targeted therapies for RC disease. *Mitochondrial cocktails* consisting of combinatorial regimens of vitamins and supplements variably include vitamins (B_1, B_2, C), antioxidants (CoQ_{10}, lipoic acid, vitamin E, *N*-acetylcysteine), and metabolic modifiers (creatine, L-carnitine, L-arginine, folinic acid, taurine). Although the efficacy, toxicity, and optimal dose of these drugs are not known and have not been objectively assessed via clinical trials in human RC disease patients, they continue to be empirically prescribed in hopes of enhancing residual RC enzymatic function or quenching toxic metabolites theorized to accumulate in RC dysfunction and because of patient-based reports of improved well-being. However, provision of these therapies has often adopted a one-size-fits-all approach, ignoring the inherent variation in primary mitochondrial disease subtypes, the tissue-specific manifestations, and the major pathogenic factors, such as the predominant downstream metabolic and signaling alterations that occur in different disease subclasses (Fig. 108.4).

Although few mitochondrial diseases have efficacious therapies that have gained regulatory approval, improved molecular delineation has enabled selected therapies to advance from the theoretical, empirical, and largely ineffective stage to a promising horizon of rational, personalized, and effective interventions. An increasing number of mitochondrial disease diagnoses have interventions involving the initiation or *avoidance* of specific medications (corticosteroids, valproic acid, phenytoin, barbiturates, propofol for prolonged duration beyond 30-60 minutes, certain anesthetics, statins, β-blocking agents, amiodarone, nucleoside reverse transcriptase inhibitors), provision of cofactors or diets, and screening regimens for progressive clinical involvement of modifiable manifestations. General therapies for **Leigh syndrome** such as L-arginine and citrulline may prevent or reverse neurodevelopmental sequelae from a metabolic stroke. Nutritional therapies in these disorders are tailored to specific disease genes, such as thiamine and biotin for *SLC19A3* disease, ubiquinol for *PDSS2* (CoQ_{10} deficiency) disease, and thiamine and the ketogenic diet for *PDHA1* (pyruvate dehydrogenase) deficiency. Establishing the precise molecular diagnosis can further be lifesaving by avoiding fasting and mitochondrial-toxic medicines or general anesthetics in specific mitochondrial disease subsets, improving recurrence counseling for risk reduction of manifesting mitochondrial disease, enabling targeted screening for reported medical complications, and in some cases providing necessary cofactors or vitamins that may not otherwise have been considered. In addition, reproductive methodologies emerging in some countries for mitochondrial disease prevention, such as PGD and mitochondrial replacement technologies (MRTs), are only appropriate to consider in the setting of known pathogenic, inherited mtDNA variants.

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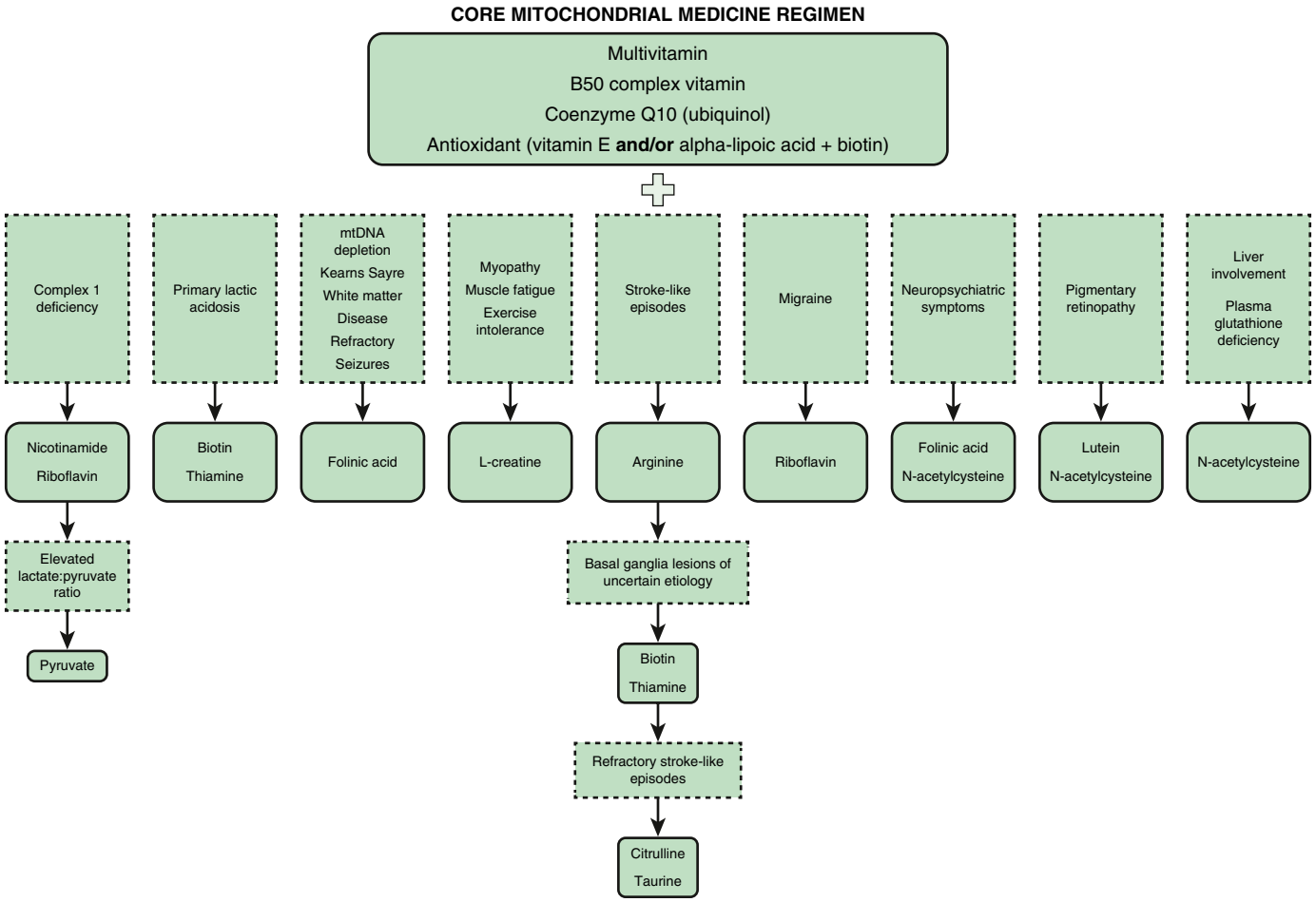

Fig. 108.4 Algorithm for the personalized prescribing of mitochondrial supplements. This figure represents the recommended approach to prescribing mitochondrial medicines. (Elevated lactate:pyruvate ratio indicates elevated NADH/NAD+ ratio.) B50, vitamins B1, B2, B3, B6, B12. (*From Barcelos I, Shadiack E, Ganetzky RD, Falk MJ. Mitochondrial medicine therapies: rationale, evidence, and dosing guidelines. Curr Opin Pediatr. 2020;32[6]:707–718.*)

Chapter 109

Mucopolysaccharidoses

Christina Lampe

Mucopolysaccharidoses are hereditary progressive diseases caused by pathogenic variants of genes coding for the lysosomal enzymes needed to degrade glycosaminoglycans (acid mucopolysaccharides). *Glycosaminoglycans* (GAGs) are long-chain complex carbohydrates composed of uronic acids, amino sugars, and neutral sugars. The major GAGs are chondroitin-4-sulfate, chondroitin-6-sulfate, heparan sulfate, dermatan sulfate, keratan sulfate, and hyaluronan. These substances are synthesized and, with the exception of hyaluronan, linked to proteins to form *proteoglycans,* major constituents of the ground substance of connective tissue and of nuclear and cell membranes. Degradation of proteoglycans starts with the proteolytic removal of the protein core, followed by the stepwise degradation of the GAG moiety. Failure of this degradation because of absent or grossly reduced activity of mutated lysosomal enzymes results in the intralysosomal accumulation of GAG fragments (Fig. 109.1). Distended lysosomes accumulate in the cell, interfere with cell function, and lead to characteristic patterns of clinical, radiologic, and biochemical abnormalities (Table 109.1 and Fig. 109.2). Within these patterns, specific diseases can be recognized that evolve from the intracellular accumulation of different degradation products (Table 109.2). As a general rule, the impaired degradation of heparan sulfate is more closely associated with **intellectual disability** and that of dermatan, chondroitin, and keratan sulfate with **mesenchymal abnormalities**. Variable expression within a given entity results from allelic pathogenic variants and varying residual activity of mutated enzymes. For instance, different allelic variants of the gene encoding L-iduronidase may result in severe **Hurler disease** (Hurler syndrome) with early death or in mild **Scheie disease** (Scheie syndrome) manifesting only with limited joint mobility, mild skeletal abnormalities, and corneal opacities. In addition, the features of an individual patient will be modified by secondary effects of lysosomal dysfunction and environmental factors.

Mucopolysaccharidoses are autosomal recessive disorders, with the exception of **Hunter disease** (Hunter syndrome), which is X-linked recessive. Their birth prevalence varies between 1.2 per 100,000 births (United States) and 16.9 per 100,000 births (Saudi Arabia). In the United States the most common subtype is MPS-III, followed by MPS-I and MPS-II.

CLINICAL ENTITIES

Mucopolysaccharidosis I

Mucopolysaccharidosis I (MPS-I) is caused by pathogenic variants of the *IUA* gene on chromosome 4p16.3 encoding α-L-iduronidase. Two major alleles, W402X and Q70X, account for more than half of the MPS-I alleles in the White population. Other pathogenic variants typically occur in only one or a few individuals.

Deficiency of α-L-iduronidase results in a wide range of clinical involvement. Homozygous nonsense pathogenic variants result in the absence of enzyme and severe forms of MPS-I (Hurler syndrome). In contrast, missense pathogenic variants are more likely to preserve some residual enzyme activity and be associated with a milder form of the disease, including the milder Scheie syndrome.

Hurler Disease

The Hurler form of MPS-I **(MPS I-H)** is a severe, progressive disorder with involvement of multiple organ and tissue involvement that results in premature death, usually by 10 years of age. An infant with Hurler syndrome appears normal at birth, but inguinal hernias may be present. Diagnosis is usually made at 6-24 months, with evidence of hepatosplenomegaly, coarse facial features, corneal clouding, large tongue, enlarged head circumference, joint stiffness, short stature, and skeletal dysplasia. Acute cardiomyopathy has been found in some

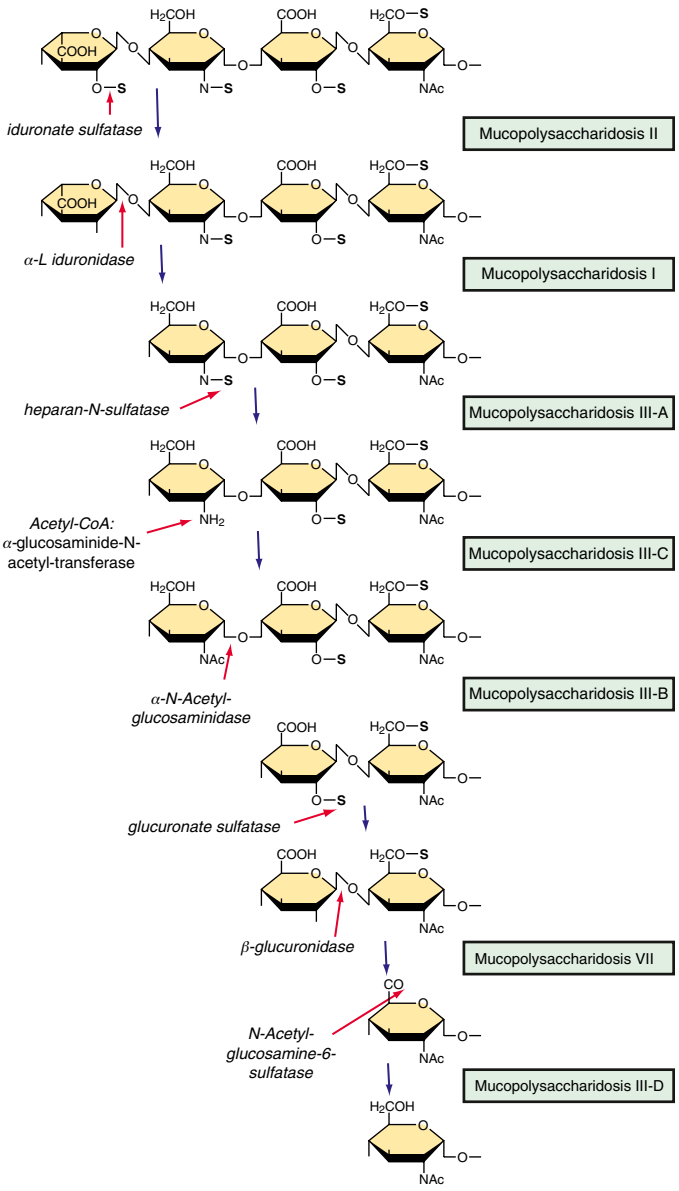

Fig. 109.1 Degradation of heparan sulfate and mucopolysaccharidoses resulting from the deficiency of individual enzymes. Some of the enzymes are also involved in the degradation of other glycosaminoglycans (not shown).

infants <1 year. Most patients have recurrent upper respiratory tract and ear infections, noisy breathing, and persistent copious nasal discharge. Valvular heart disease, notably with incompetence of the mitral and aortic valves, regularly develops, and narrowing of the coronary arteries occurs. Obstructive airway disease, especially during sleep, may necessitate tracheotomy. Obstructive airway disease, respiratory infection, and cardiac complications are the common causes of death.

Most children with Hurler syndrome acquire only limited language skills because of developmental delay, combined conductive and neurosensory hearing loss, and an enlarged tongue. Progressive ventricular enlargement with increased intracranial pressure caused by communicating hydrocephalus also occurs. Corneal clouding, glaucoma, and retinal degeneration are common. Radiographs show a characteristic skeletal dysplasia known as *dysostosis multiplex* (Figs. 109.3 and 109.4). The earliest radiographic signs are thick ribs and ovoid vertebral bodies. Skeletal abnormalities (in addition to those shown in the figures) include enlarged, coarsely trabeculated diaphyses of the long bones with irregular metaphyses and epiphyses. With progression of disease, macrocephaly develops with thickened calvarium, premature closure

of lambdoid and sagittal sutures, shallow orbits, enlarged J-shaped sella, and abnormal spacing of teeth with dentigerous cysts.

Hurler-Scheie Disease

The clinical phenotype of the Hurler-Scheie form of MPS-I **(MPS I-H/S)** is intermediate between Hurler and Scheie diseases and is characterized by progressive somatic involvement, including dysostosis multiplex with little or no intellectual dysfunction. The onset of symptoms is usually observed between 3 and 8 years of age, and survival to adulthood is common. Cardiac involvement and upper airway obstruction contribute to clinical morbidity. Some patients have spondylolisthesis, which may cause cord compression.

Scheie Syndrome

MPS I-S is a comparatively mild disorder characterized by joint stiffness, aortic valve disease, corneal clouding, and mild dysostosis multiplex. Onset of significant symptoms is usually after age 5 years, with diagnosis made between 10 and 20 years. Patients with Scheie syndrome have normal intelligence and stature but have significant joint and ocular involvement. A carpal tunnel syndrome often develops. Ophthalmic features include corneal clouding, glaucoma, and retinal degeneration. Obstructive airway disease, causing sleep apnea, develops in some patients, necessitating tracheotomy. Aortic valve disease is common and has required valve replacement in some patients.

Table 109.1 Recognition Pattern of Mucopolysaccharidoses

	MUCOPOLYSACCHARIDOSIS (MPS) TYPE						
MANIFESTATIONS	I-H	I-S	II	III	IV	VI	VII
Intellectual disability	+	−	±	+	−	−	±
Coarse facial features	+	(+)	+	+	−	+	±
Corneal clouding	+	+	−	−	(+)	+	±
Visceromegaly	+	(+)	+	(+)	−	+	+
Short stature	+	(+)	+	−	+	+	+
Joint contractures	+	+	+	−	−	+	+
Dysostosis multiplex	+	(+)	+	(+)	+	+	+
Leucocyte inclusions	+	(+)	+	+	−	+	+
Mucopolysacchariduria	+	+	+	+	+	+	+

I-H, Hurler syndrome; I-S, Scheie syndrome; II, Hunter syndrome; III, Sanfilippo syndrome; IV, Morquio syndrome; VI, Maroteaux-Lamy syndrome; VII, Sly syndrome, +, presence of manifestation, −, absence of manifestation; ±, possible presence of manifestation; (+), mild manifestation.

Mucopolysaccharidosis II

Hunter disease (MPS-II) is an X-linked disorder caused by the deficiency of iduronate 2-sulfatase (IDS). The gene encoding IDS is located on Xq28. Single-nucleotide variants or small deletions of *IDS* have been detected in approximately 50% of patients with MPS-II. Major deletions or rearrangements of *IDS* have been found in the rest, usually associated with a more severe and complex clinical phenotype. As an X-linked recessive disorder, Hunter syndrome manifests almost exclusively in males. However, it has been observed in females because of unfavorable skewing of inactivation of the normal X chromosome carrying the normal gene.

Marked molecular heterogeneity explains the wide clinical spectrum of Hunter disease. Patients with severe MPS-II have features similar to those of Hurler disease, except for the lack of corneal clouding and the somewhat slower progression of somatic and central nervous system (CNS) deterioration. Coarse facial features, short stature, dysostosis multiplex, joint stiffness, and intellectual disability manifest between 2 and 4 years of age. Grouped skin papules are present in some patients. Extensive congenital dermal melanocytosis (Mongolian spots) present at birth have been observed in African and Asian patients and may be an early marker of the disease. Gastrointestinal (GI) storage may produce chronic diarrhea. Communicating hydrocephalus and spastic paraplegia may develop due to thickened meninges. In severely affected patients, extensive, slowly progressive neurologic involvement precedes death, which usually occurs at age 10-15 years.

Patients with the mild form have a near-normal or normal life span, minimal CNS involvement, and slow progression of somatic deterioration with preservation of cognitive function in adult life. Survival to ages 65 and 87 years has been reported, and some patients have had children. Somatic features are Hurler-like but milder with a greatly

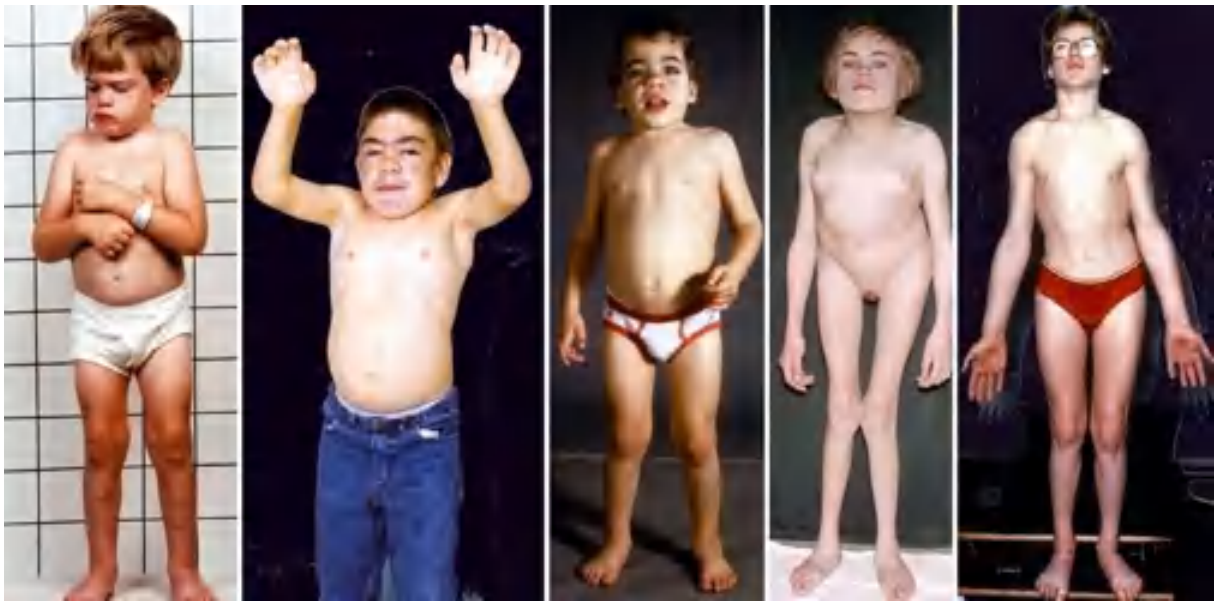

Fig. 109.2 Patients with various types of mucopolysaccharidoses. MPS-I: Hurler syndrome, age 3 years; MPS-II: Hunter syndrome, 12 years; MPS-III: Sanfilippo syndrome, 4 years; MPS-IV: Morquio syndrome, 10 years; MPS-VI: Maroteaux-Lamy syndrome, 15 years.

Table 109.2 Mucopolysaccharidoses: Clinical, Molecular, and Biochemical Aspects

MPS TYPE	EPONYM	INHERITANCE	GENE/ CHROMOSOME	MAIN CLINICAL FEATURES	DEFECTIVE ENZYME	ASSAY	MIM NUMBER
I-H	(Pfaundler-) Hurler	AR	*IDUA* 4p16.3	Severe Hurler phenotype, mental deficiency, corneal clouding, death usually before age 14 yr	α-L-iduronidase	L, F, Ac, Cv	252800 607014
I-S	Scheie	AR	*IDUA* 4p16.4	Stiff joints, corneal clouding, aortic valve disease, normal intelligence, survive to adulthood	α-L-iduronidase	L, F, Ac, Cv	607016
I-HS	Hurler-Scheie	AR	*IDUA* 4p16.4	Phenotype intermediate between I-H and I-S	α-L-iduronidase	L, F, Ac, Cv	607015
II	Hunter	XLR	*IDS* Xq27.3-28	Severe course: similar to I-H but clear corneas Mild course: less pronounced features, later manifestation, survival to adulthood with mild mental deficiency or without mental deficiency	Iduronate sulfate sulfatase	S, F, Af, Ac, Cv	309900
IIIA	Sanfilippo A	AR	*SGSH* 17q25.3	Behavioral problems, sleeping disorder, aggression, progressive dementia, mild dysmorphism, coarse hair, clear corneas Survival to adulthood possible	Heparan-S-sulfamidase	L, F, Ac, Cv	252900 605270
IIIB	Sanfilippo B	AR	*NAGLU* 17q21		*N*-Acetyl-D-glycosaminidase	S, F, Ac, Cv	252920
IIIC	Sanfilippo C	AR	*HGSNAT* 8p11.21		Acetyl-CoA-glucosaminide *N*-acetyltransferase	F, Ac	252930
IIID	Sanfilippo D	AR	*GNS* 12q14		*N*-Acetylglucosamine–6-sulfate sulfatase	F, Ac	252940 607664
IVA	Morquio A	AR	*GALNS* 16q24.3	Short-trunk dwarfism, fine corneal opacities, characteristic bone dysplasia; final height <125 cm	*N*-Acetylgalactosamine-6-sulfate sulfatase	L, F, Ac	253000
IVB	Morquio B	AR	*GLB1* 3p21.33	Same as IVA, but milder; adult height >120 cm	β-Galactosidase	L, F, Ac, Cv	253010 230500
VI	Maroteaux-Lamy	AR	*ARSB* 5q11-q13	Hurler phenotype with marked corneal clouding but normal intelligence; mild, moderate, and severe expression in different families	*N*-Acetylgalactosamine-α-4-sulfate sulfatase (arylsulfatase B)	L, F, Ac	253200
VII	Sly	AR	*GUSB* 7q21.11	Varying from fetal hydrops to mild dysmorphism; dense inclusions in granulocytes	β-Glucuronidase	S, F, Ac, Cv	253220
IX	Hyaluronidase deficiency	AR	*HYAL1* 3p21.3	Periarticular masses, no Hurler phenotype	Hyaluronidase 1	S	601492
MPSPS	MPS plus syndrome	AR	*VPS33A*	Mild Hurler phenotype, cognitive deficiency, organomegaly, skeletal dysplasia, pancytopenia, renal insufficiency, optic atrophy, early death	No lysosomal enzyme deficiency	L, F	617303

AR, Autosomal recessive; XLR, X-linked recessive; L, Leukocytes; S, serum; F, cultured fibroblasts; Ac, cultured amniotic cells; Af, amniotic fluid; Cv, chorionic villus sampling; MIM, Mendelian Inheritance in Man Catalogue.

reduced rate of progression. Adult height may exceed 150 cm. Airway involvement, valvular cardiac disease, hearing impairment, carpal tunnel syndrome, and joint stiffness are common and can result in significant loss of function in both the mild and severe forms.

Mucopolysaccharidosis III

Sanfilippo disease makes up a genetically heterogeneous but clinically similar group of four recognized types. Each type is caused by a different enzyme deficiency involved in the degradation of heparan sulfate (see Fig. 109.1). Pathogenic variants have been found in all the MPS-III disorders for which genes have been isolated.

Phenotypic variation exists in MPS-III patients, but to a lesser degree than in other MPS disorders. Patients with Sanfilippo disease are characterized by slowly progressive, severe CNS involvement with mild somatic disease. Such disproportionate involvement of the CNS versus the connective tissue system is unique to MPS-III. Onset of clinical features usually occurs at age 2-6 years in a child who previously appeared normal. Presenting features include delayed development,

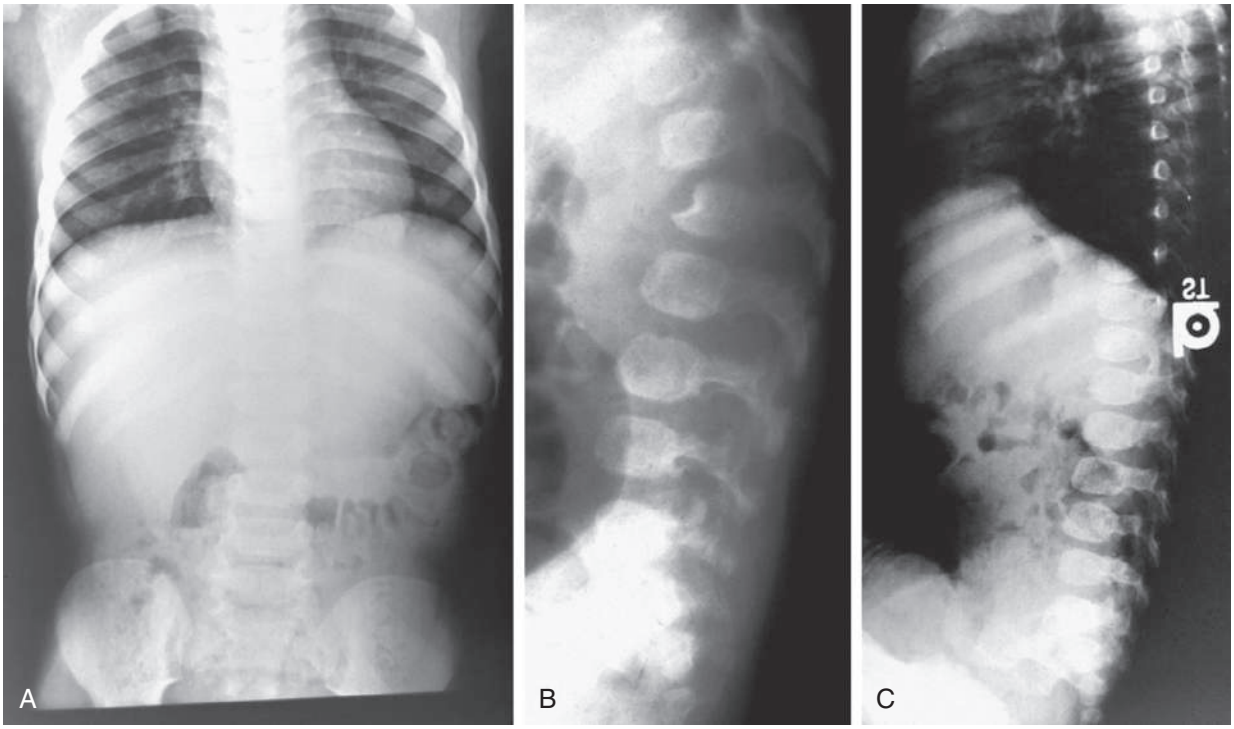

Fig. 109.3 Dysostosis multiplex. **A,** Sanfilippo syndrome, patient age 4 years; the ribs are wide. **B,** Sanfilippo syndrome, age 4 years; immature, ovoid configuration of the vertebral bodies. **C,** Hurler syndrome, age 18 months; anterosuperior hypoplasia of first lumbar vertebra (L1) resulting in hook-shaped appearance.

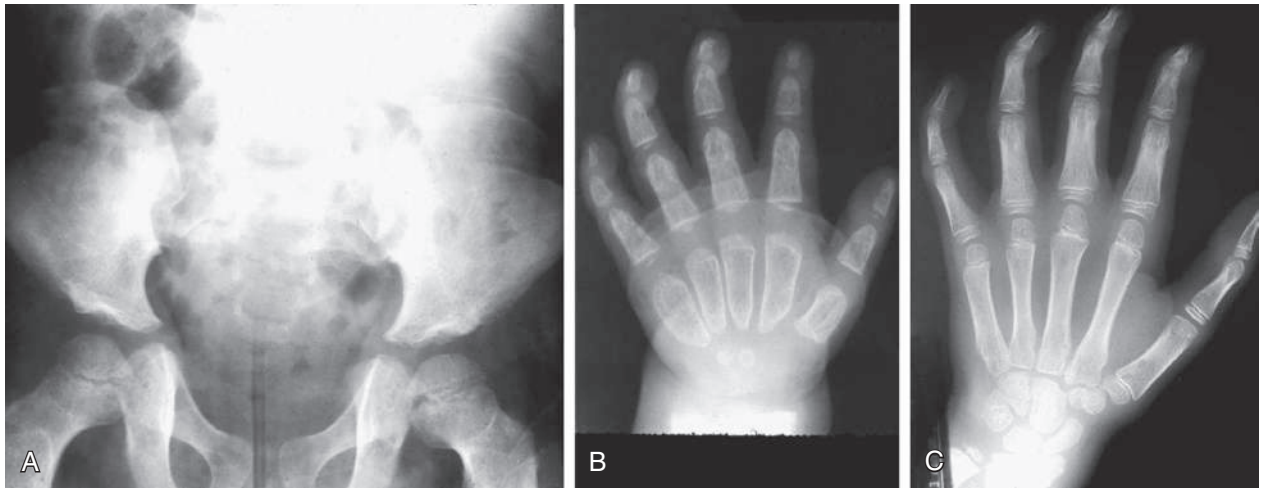

Fig. 109.4 Dysostosis multiplex. **A,** Mucopolysaccharidosis I-H, patient age 10 years. The inferior portions of the ilia are hypoplastic, with resulting iliac flare and shallow acetabular fossae. The femoral necks are in the valgus position. **B,** MPS I-H, age 4 years. Metacarpals and phalanges are abnormally short, wide, and deformed with proximal pointing of the metacarpals and bullet-shaped phalanges. Bone trabeculation is coarse, and the cortices are thin. **C,** MPS I-S, age 13 years. The carpal bones are small, leading to a V-shaped configuration of the digits. The short, tubular bones are well modeled. Flexion of the middle and distal phalanges II-V is caused by joint contractures.

hyperactivity with aggressive behavior, coarse hair, hirsutism, sleep disorders, and mild hepatosplenomegaly. Subclinical cardiac defects are common. Delays in the diagnosis of MPS-III are common because of mild physical features, hyperactivity, and slowly progressive neurologic disease. Severe neurologic deterioration occurs in most patients by age 6-10 years, accompanied by rapid deterioration of social and adaptive skills. Severe behavior problems, such as sleep disturbance, uncontrolled hyperactivity, temper tantrums, destructive behavior, and physical aggression, are common. Profound developmental regression and behavior problems often occur in patients with normal physical strength, making management particularly difficult.

Mucopolysaccharidosis IV

Morquio disease (MPS-IV) is caused by a deficiency of *N*-acetylgalactosamine-6-sulfatase (MPS-IVA) or of β-galactosidase (MPS-IVB). Both result in the defective degradation of keratan sulfate. The gene encoding *N*-acetylgalactosamine-6-sulfatase is *GALNS* on chromosome 16q24.3, and the gene encoding β-galactosidase is *GLB1* on chromosome 3p21.33. β-Galactosidase catalyzes GM_1 ganglioside in addition to endohydrolysis of keratan sulfate, and most pathogenic variants of *GLB1* result in generalized **gangliosidosis**, a spectrum of neurodegenerative disorders associated with dysostosis multiplex. A W273L pathogenic variant of the *GLB1* gene, either in the homozygous

state or as part of compound heterozygosity, commonly results in Morquio B disease.

Both types of Morquio syndrome are characterized by short-trunk dwarfism, fine corneal deposits, a skeletal dysplasia that is distinct from other mucopolysaccharidoses, and preservation of intelligence. MPS-IVA is usually more severe than MPS-IVB, with an adult height of <125 cm in the former and more than 150 cm in the latter. However, there is considerable variability of expression in both subtypes. The appearance of genu valgum, kyphosis, growth retardation with short trunk and neck, and waddling gait with a tendency to fall are early symptoms of MPS-IV. Extraskeletal manifestations include mild corneal clouding, small teeth with abnormally thin enamel, frequent caries formation, and occasionally hepatomegaly and cardiac valvular lesions. Instability of the odontoid process and ligamentous laxity is regularly present and can result in atlantoaxial instability and life-threatening dislocation. Surgery to stabilize the upper cervical spine, usually by posterior spinal fusion, before the development of cervical myelopathy, can be lifesaving.

Mucopolysaccharidosis VI

Maroteaux-Lamy disease (MPS-VI) is caused by pathogenic variants of the *ARSB* gene on chromosome 5q11-13 encoding *N*-acetylgalactosamine-4-sulfatase (arylsulfatase B). It is characterized by severe to mild somatic involvement, as seen in MPS-I, but with preservation of intelligence. The somatic involvement of the severe form of MPS VI is characterized by corneal clouding, coarse facial features, joint stiffness, valvular heart disease, communicating hydrocephalus, and dysostosis multiplex. In the severe form, growth can be normal for the first few years of life but seems to virtually stop after age 6-8 years. The mild to intermediate forms of Maroteaux-Lamy syndrome can be easily confused with Scheie syndrome. Spinal cord compression from thickening of the dura in the upper cervical canal with resultant myelopathy is common in patients with MPS-VI.

Mucopolysaccharidosis VII

Sly disease (MPS-VII) is caused by pathogenic variants of the *GUSB* gene. Pathogenic variants result in a deficiency of β-glucuronidase, intracellular storage of glycosaminoglycan fragments, and a very wide range of clinical involvement. The most severe form presents as lethal nonimmune fetal hydrops and may be detected in utero by ultrasound. Some severely affected newborns survive for months and have, or develop, signs of lysosomal storage, including thick skin, visceromegaly, and dysostosis multiplex. Less severe forms of MPS-VII present during the first years of life with features of MPS-I but slower progression. Corneal clouding varies. Patients with manifestation after 4 years of life have coarse facial features with depressed nasal roots, malpositioned teeth with malformed roots, and skeletal abnormalities of dysostosis multiplex. Intelligence is normal, and corneae are usually clear. Patients may be found incidentally based on a blood smear that shows coarse granulocytic inclusions.

Mucopolysaccharidosis IX

MPS-IX disease is caused by pathogenic variants in *HYAL1* encoding one of three hyaluronidases. Clinical findings in the first patient, a 14-year-old girl, were bilateral nodular soft tissue periarticular masses, lysosomal storage of histiocytes, mildly dysmorphic craniofacial features, short stature, normal joint movement, and normal intelligence. Clinical findings in the only three additional patients known today were indistinguishable from those in rheumatoid arthritis.

Mucopolysaccharidosis Plus Syndrome

Coarse facial features, organomegaly, joint contractures, dysostosis multiplex, cognitive deficiency, increased mucopolysacchariduria, and massive intracellular accumulation of heparan sulfate have been found in 13 children in northeastern Siberia and two Turkish children. Additional findings include optic atrophy, intracerebral calcifications, pancytopenia, and renal insufficiency. Most patients died within the first 2 years of life from cardiorespiratory failure. Lysosomal enzyme activities were normal in children with MPS plus syndrome. This autosomal recessive multisystem disorder is caused by biallelic pathogenic variants of *VPS33A* encoding a protein involved in lysosomal fusion processes.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Clinical suspicion of an MPS justifies a skeletal survey. Radiographs of the chest, spine, pelvis, and hands may show early signs of dysostosis multiplex. The next diagnostic step is to assay the urinary excretion of GAGs. Semiquantitative spot tests for increased urinary GAG excretion are quick, inexpensive, and useful for initial evaluation but are subject to both false-positive and false-negative results. Quantitative analysis of a single GAG in material derived from dried blood spots is preferable with few false-negative results. Using tandem mass spectrometry, type-specific profiles can be obtained. Morquio disease is often missed in urinary assays but can be reliably diagnosed in serum using monoclonal antibodies to keratan sulfate.

Newborn screening for MPS (type I, II, VI) is essential for the early detection of subclinical cases and their therapy. It is available from dried blood spots using tandem mass spectrometry of glycosaminoglycans or fluorescence-based enzyme function assays. Any individual with a suspected MPS disorder based on screening tests, clinical features, and radiographic results should have a definitive diagnosis established by enzyme assay. Serum, leukocytes, or cultured fibroblasts are used as the tissue source for measuring lysosomal enzymes (see Table 109.2).

Prenatal diagnosis is available for all MPSs and is carried out on cultured cells from amniotic fluid or chorionic villus biopsy. Molecular analysis is typically performed using appropriate gene panels. In many cases the type and location of the pathogenic variant are related to the future course of the disease and thus have a prognostic value. Carrier testing in Hunter syndrome, an X-linked disorder, requires analysis of *IDS* once the specific pathogenic variant or chromosome arrangement in the family is known. Prenatal molecular analysis should be offered in a male fetus of a proven female carrier of the *IDS* gene. His risk to be affected is 50%. In a female fetus, the risk is small, but not zero, as a result of skewed maternal X chromosome inactivation.

Mucolipidoses and oligosaccharidoses manifest with the same clinical and radiographic features as MPS. In these conditions the urinary excretion of GAGs is not elevated. Hurler-like facial features, joint contractures, dysostosis multiplex, and elevated urinary GAG excretion differentiate the MPSs from congenital disorders of glycosylation and other neurodegenerative and dwarfing conditions.

TREATMENT

Hematopoietic stem cell transplantation and enzyme replacement therapy are performed in specialized institutions.

Bone marrow transplantation from related or unrelated donors and cord blood transplantation have resulted in significant clinical improvement of somatic disease in MPSs I, II, III, and VII. Clinical effects include increased life expectancy with resolution or improvement of growth, hepatosplenomegaly, joint stiffness, facial appearance, skin changes, obstructive sleep apnea, heart disease, communicating hydrocephalus, and hearing loss. Enzyme activity in serum and urinary GAG excretion normalize. This is true for MPS I-H, II, and III. Some patients with MPS-I who have undergone transplantation before 9 months of age have been reported with normal cognitive development. Transplantation before 24 months and with a baseline mental development index >70 have improved long-term outcomes. Transplantation does not significantly improve the neuropsychologic outcome of MPS patients with impaired cognition at transplantation. Early transplantation in the MPS-II patient may have the same effect. Transplantation in the MPS-VI patient stabilizes or improves cardiac manifestations, posture, and joint mobility. Stem cell transplantation does not correct skeletal or ocular anomalies, and they should be treated with appropriate orthopedic and ophthalmologic procedures. Cord blood transplantation is the therapy of choice in children with MPS-IH, and possibly MPS-II, before the age of 2 years, but transplantation-related death or primary graft failure, which occurs in approximately one third of the patients, must be weighed against other therapeutic options.

Enzyme replacement therapy (ERT) using recombinant α-L-iduronidase has been approved for patients with MPS-I (Table 109.3). It reduces organomegaly and airway infections, improves rate of growth, improves joint mobility, pulmonary function, and endurance, and reduces the number of episodes of sleep apnea and urinary GAG excretion. The enzyme does not cross the blood-brain barrier and does not prevent deterioration of cognition and other neurologic functions. Consequently, ERT is reserved for patients with mild CNS involvement. To stabilize extraneural manifestations it is also recommended in young patients before stem cell transplantation. ERT also ameliorates somatic complications in patients with more severe CNS involvement. Recombinant iduronate-2-sulfatase ameliorates the nonneural manifestations of Hunter disease, such as respiratory dysfunction, reduced physical endurance, and daily activity. ERT with recombinant human *GALNS* improves physical endurance, respiratory function, and daily living activity of patients with MPS-IV. Similar effects produce recombinant *N*-acetylgalactosamine-4-sulfatase in patients with MPS-VI and recombinant β-glucuronidase in MPS VII.

Advanced therapies include enzyme replacement mediated via insulin receptors in MPS I and II or transferrin receptors in MPS II to assist in crossing the blood-brain barrier. A phase I/II and a phase III study using the transferrin receptor have started for MPS II. In MPS IIIA, an intravenous ERT phase 1/2 study with a chemically modified variant of recombinant human sulfamidase and a recombinant human alpha-*N*-acetylglucosaminidase for MPS IIIB were recently completed. However, the study results were not convincing. A phase I/II study for MPS III using the transferrin receptor is in planning. Gene therapies for MPS I, II, and III are under investigation. Autologous hematopoietic stem/progenitor cells transduced ex vivo with an α-L-iduronidase–encoded lentiviral vector has resulted in beneficial metabolic correction in peripheral and central nervous system tissues. A clinical trial to ameliorate the intracellular inflammation and, consequently, the CNS involvement in MPS III with anakinra (Kineret) was completed with results pending.

Symptomatic therapy focuses on respiratory and cardiovascular complications, hearing loss, carpal tunnel syndrome, spinal cord compression, hydrocephalus, and other problems (Table 109.4). The

Table 109.3 Therapies For Mucopolysaccharidoses

MPS TYPE	HEMATOPOIETIC STEM CELL TRANSPLANTATION	ENZYME REPLACEMENT THERAPY	REMARKS
I	Yes	Laronidase (Aldurazyme)	Developmental trajectory dependent on time of transplantation. Little effect on connective tissue manifestations. Enzyme replacement immediately after diagnosis. Autologous hematopoietic stem/progenitor cells transduced ex vivo with gene encoding lentiviral vector.
II	Yes	Idursulfase (Elaprase)	
III	No	No	Experimental intraventricular chimeric fusion of recombinant human N-acetyl-D-glucosaminidase and truncated insulin-like growth factor 2 in MPS IIIB
IV	Yes	Elosulfase (Vimizim)	Improved daily activities. No effect on growth or skeletal dysplasia.
VI	Yes	Galsulfase (Naglazyme)	Improved daily activities. Improved growth. No effect on skeletal dysplasia.
VII	Yes	Vestronidase alfa (MEPSEVII)	Improved daily activity. No effect on skeletal dysplasia.

Table 109.4 Symptomatic Management of Mucopolysaccharidoses

PROBLEM	PREDOMINANTLY IN	MANAGEMENT
NEUROLOGIC		
Hydrocephalus	MPS I, II, VI, VII	Fundoscopy, CT scan
Chronic headaches	All	Ventriculoperitoneal shunting
Behavioral disturbance	MPS-III	Behavioral medication, sometimes CT scan, ventriculoperitoneal shunting
Disturbed sleep–wake cycle	MPS-III	Melatonin
Seizures	MPS I, II, III	EEG, anticonvulsants
Atlantoaxial instability	MPS IV	Cervical MRI, upper cervical fusion
Spinal cord compression	All	Laminectomy, dural excision
OPHTHALMOLOGIC		
Corneal opacity	MPS I, VI, VII	Corneal transplant
Glaucoma	MPS I, VI, VII	Medication, surgery
Retinal degeneration	MPS I, II	Nightlight
EARS, AIRWAYS		
Recurrent otitis media	MPS I, II, VI, VII	Ventilating tubes
Impaired hearing	All except MPS-IV	Audiometry, hearing aids
Obstruction	All except MPS-III	Adenectomy, tonsillectomy, bronchodilator therapy, CPAP at night, laser excision of tracheal lesions, tracheotomy
CARDIAC		
Cardiac valve disease	MPS I, II, VI, VII	Endocarditis prevention, valve replacement
Coronary insufficiency	MPS I, II, VI, VII	Medical therapy
Arrhythmias	MPS I, II, VI, VII	Antiarrhythmic medication, pacemaker

Table 109.4 Symptomatic Management of Mucopolysaccharidoses—cont'd

PROBLEM	PREDOMINANTLY IN	MANAGEMENT
ORAL, GASTROINTESTINAL		
Hypertrophic gums, poor teeth	MPS I, II, VI, VII	Dental care
Chronic diarrhea	MPS-II	Diet modification, loperamide
MUSCULOSKELETAL		
Joint stiffness	All except MPS IV	Physical therapy
Scoliosis	All	Bracing, surgery
Weakness	All	Physical therapy, wheelchair
Gross long-bone malalignment	All	Corrective osteotomies
Carpal tunnel syndrome	MPS I, II, VI, VII	Electromyography, surgical decompression
ANESTHESIA	All except MPS III	Avoid atlantoaxial dislocation; use angulated video intubation laryngoscope and small endotracheal tubes

CT, Computed tomography; CPAP, continuous positive airway pressure; EEG, electroencephalogram; MRI, magnetic resonance imaging.

multisystem involvement and progressive nature of MPS syndromes usually require the standardized and complex care provided by medical centers.

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Chapter 110

Disorders of Purine and Pyrimidine Metabolism

H. A. Jinnah

The purines and pyrimidines consist of a family of small organic molecules that are found in all cells. They are often considered together because they provide the fundamental building blocks for nucleic acids, which include DNA and RNA. The purines and pyrimidines are also involved in numerous other cellular processes. Although they share certain biochemical characteristics, the associated clinical disorders are quite varied. Historically, most were identified by characteristic abnormalities found with metabolic tests. Currently, they are increasingly being identified after discovery of a pathogenic variant in a relevant gene in tests that involve broad gene panels or exome sequencing. Most of these disorders are genetic, and most are rare. *However, they are important to recognize because specific treatments are available for some of them* (Table 110.1).

PURINES

The purines are divided into two groups. The first group involves the base adenine, its corresponding nucleoside (adenosine), and nucleotide (AMP, ADP, ATP) derivatives (Fig. 110.1). ATP serves as one of four building blocks for RNA, and its deoxy derivative (dATP) serves as one of four building blocks for DNA. ATP also provides the energy source for the majority of energy-requiring activities. ATP derivatives act as signaling factors as "second messengers" (cAMP) or cofactors (FAD, NAD, NADP) for a large number of cellular processes. Some purines such as adenosine and the adenine nucleotides serve as extracellular signaling molecules, including acting as neurotransmitters in the nervous system.

The second group of purines involves the base guanine, its corresponding nucleoside (guanosine), and nucleotide (GMP, GDP, GTP) derivatives (see Fig. 110.1). Like the adenine nucleotides, guanine nucleotide derivatives also serve as building blocks for nucleic acids (DNA and RNA). GTP also provides an energy source for some energy-requiring activities. Guanine nucleotides also play a critical role in intercellular signaling (cGMP) via membrane-bound receptors that require GTP during signal transduction (G-proteins). They also play a role in intracellular signaling via a large family of cytosolic GTPases and guanine nucleotide exchange factors.

Because purines play a critical role for so many fundamental cellular processes, their amounts are tightly regulated. The amounts of purines reflect a balance between synthesis, catabolism, and recycling (Fig. 110.2). Purines are synthesized by the de novo purine synthesis pathway. This pathway has six enzymes that carry out 10 reactions. When there is a demand for more purines, these six enzymes aggregate dynamically into giant macromolecular complexes known as *purinosomes* to accelerate synthesis. After the demand is met, these aggregates disperse and synthesis abates. In humans, purines are catabolized via several routes. The major end product of purine catabolism is uric acid, produced by the enzyme encoded by the *XDH* gene. The enzyme is known as *xanthine oxidase, xanthine reductase,* or *xanthine oxidoreductase.* Most of this uric acid is eliminated from the body in the urine, although some is also eliminated by secretion into the gastrointestinal tract. Because the synthesis of purines is energetically costly, purines are actively recycled (see Fig. 110.2). The key enzymes of purine salvage include hypoxanthine-guanine phosphoribosyl transferase (encoded by *HPRT1*) and adenine phosphoribosyl transferase (encoded by *APRT*).

The metabolism of purines differs considerably across different cells and tissues. For example, the synthetic pathway is absent in erythrocytes, because these postmitotic cells do not require large quantities of new purines to make nucleic acids for cell division. Erythrocytes rely instead predominantly on purine salvage to maintain purine levels. A similar situation is thought to occur for postmitotic neurons of the nervous system. Another example of tissue-specific differences in purine metabolism involves uric acid. The vast majority of uric acid in the human body is produced by the liver, because hepatocytes express high levels of *XDH,* whereas many other cells and tissues express little or none. In addition to tissue differences, several enzymes of purine metabolism have multiple isoforms with different tissue distributions, leading to tissue-specific consequences in disorders where one isoform is affected.

PYRIMIDINES

The pyrimidines are divided into three groups delineated by the bases cytosine, thymine, and uracil (see Fig. 110.1). Each has a corresponding nucleoside derivative (cytidine, thymidine, uridine) and nucleotide

Table 110.1 Disorders of Purines and Pyrimidines with Specific Treatments

DISORDER	GENE SYMBOL	TREATMENT	OUTCOME
APRT deficiency	*APRT*	XOR inhibitor	Prevents renal stones and renal failure
Uridine-responsive epileptic encephalopathy	*CAD*	Uridine	Suppresses seizures, reverses neurobehavioral and hematologic abnormalities
Familial juvenile hyperuricemic nephropathy	*UMOD, REN, MUC,* or *HNF-1b*	XOR inhibitor	Prevents renal stones, renal failure, and gout
Renal hypouricemia	*SLC2A9* or *SLC22A12*	XOR inhibitor	Prevents renal stones and renal failure
Hereditary orotic aciduria	*UMPS*	Uridine	Reverses neurobehavioral and hematologic deficits
Hereditary xanthinurias			
Type 1	*XOR*	XOR inhibitor	Prevents renal stones and renal failure
Type 2	*MOCOS*	XOR inhibitor	Prevents renal stones and renal failure
Molybdenum cofactor deficiencies	*MOCS1-3, GPHN*	XOR inhibitor	Prevents renal stones and renal failure
MOCS1 deficiency	*MOCS1*	cPMP	Reverses neurobehavioral deficits
HPRT1-associated disorders	*HPRT1*	XOR inhibitor	Prevents renal stones, renal failure, and gout
PRPP synthetase hyperactivity	*PRPS1*	XOR inhibitor	Prevents renal stones, renal failure, and gout
Nucleotidase-associated pervasive developmental delay	Unknown	Uridine	Reverses neurobehavioral deficits

The most common XOR inhibitors include allopurinol, febuxostat, and topiroxostat.
cPMP, Cyclic pyranopterin monophosphate; XOR, xanthine oxidoreductase.

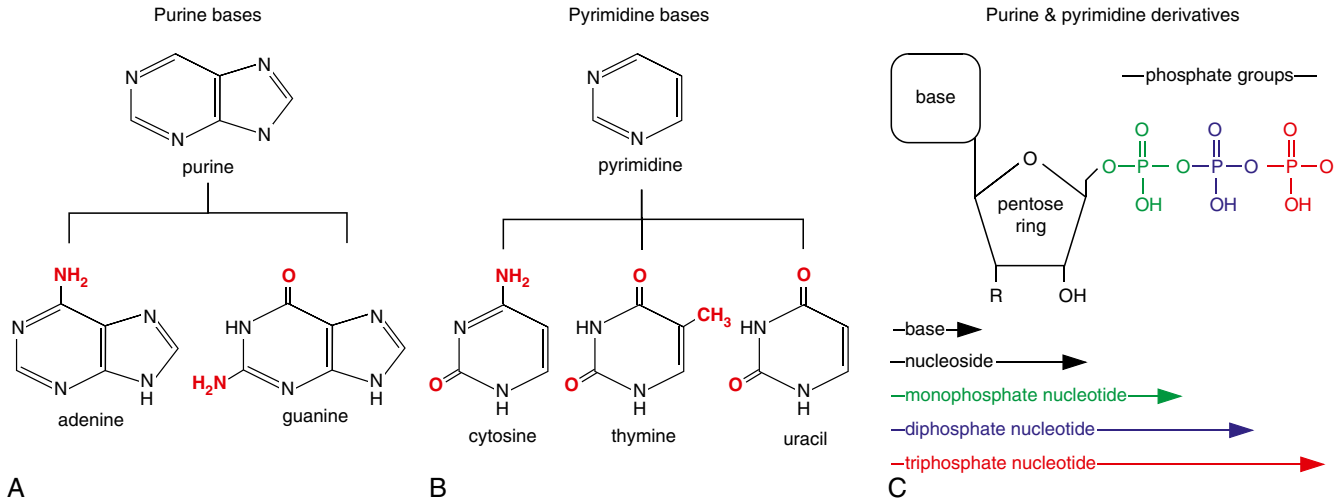

Fig. 110.1 The structure of purines and pyrimidines. A, The fundamental purine base and its variants adenine and guanine. B, The fundamental pyrimidine base and its variants cytosine, thymine, and uracil. C, The major purine and pyrimidine derivatives. Nucleosides are composed of a purine or pyrimidine base attached to a pentose ring. The R moiety in the pentose ring can be either an OH group (ribose for RNA) or a hydrogen (deoxyribose for DNA). Nucleotides are composed of a nucleoside attached to phosphate groups. (*Copyright H.A. Jinnah.*)

derivatives (see Fig. 110.1). Like the purines, the pyrimidines are key ingredients for nucleic acids. Nucleotide derivatives of cytidine are used to make DNA and RNA. Nucleotide derivatives of thymidine are used in DNA, but nucleotide derivatives of uridine replace thymidine in RNA.

The pyrimidines also have additional functions; CTP is used to make CDP-choline, an important intermediate in the production of membrane phospholipids such as phosphatidylcholine. UTP is used to make UDP-glucose, an intermediate used in the synthesis of glycogen, as well as other glycosylation reactions, to make glycolipids or glycoproteins. UTP is also used to make UDP-glucuronic acid, an intermediate used in the generation of glucuronide conjugates. Glucuronide conjugates of certain drugs and drug metabolites are excreted in the bile, and glucuronide conjugates of other endogenous biomolecules such as steroid hormones facilitate transport between tissues in the body.

The body's pyrimidine levels reflect a balance between synthesis, catabolism, and recycling. Pyrimidines are synthesized in a pathway that involves three enzymes which carry out six steps leading to the central metabolite UMP (Fig. 110.3). UMP can then be directed toward the synthesis of other pyrimidines. Pyrimidines are catabolized via several routes. Cytidine and uridine derivatives are both converted to uracil, and ultimately to β-alanine. Thymidine is converted to β-aminoisobutyrate. Both β-alanine and β-aminoisobutyrate serve as intermediates in the citric acid cycle. Free pyrimidines are also recycled at several steps by kinases that convert nucleosides to their corresponding nucleotides (see Fig. 110.3). Pyrimidine disorders are rare and clinically heterogeneous. The heterogeneity likely reflects the different functions of pyrimidines in different tissues combined with differences in the

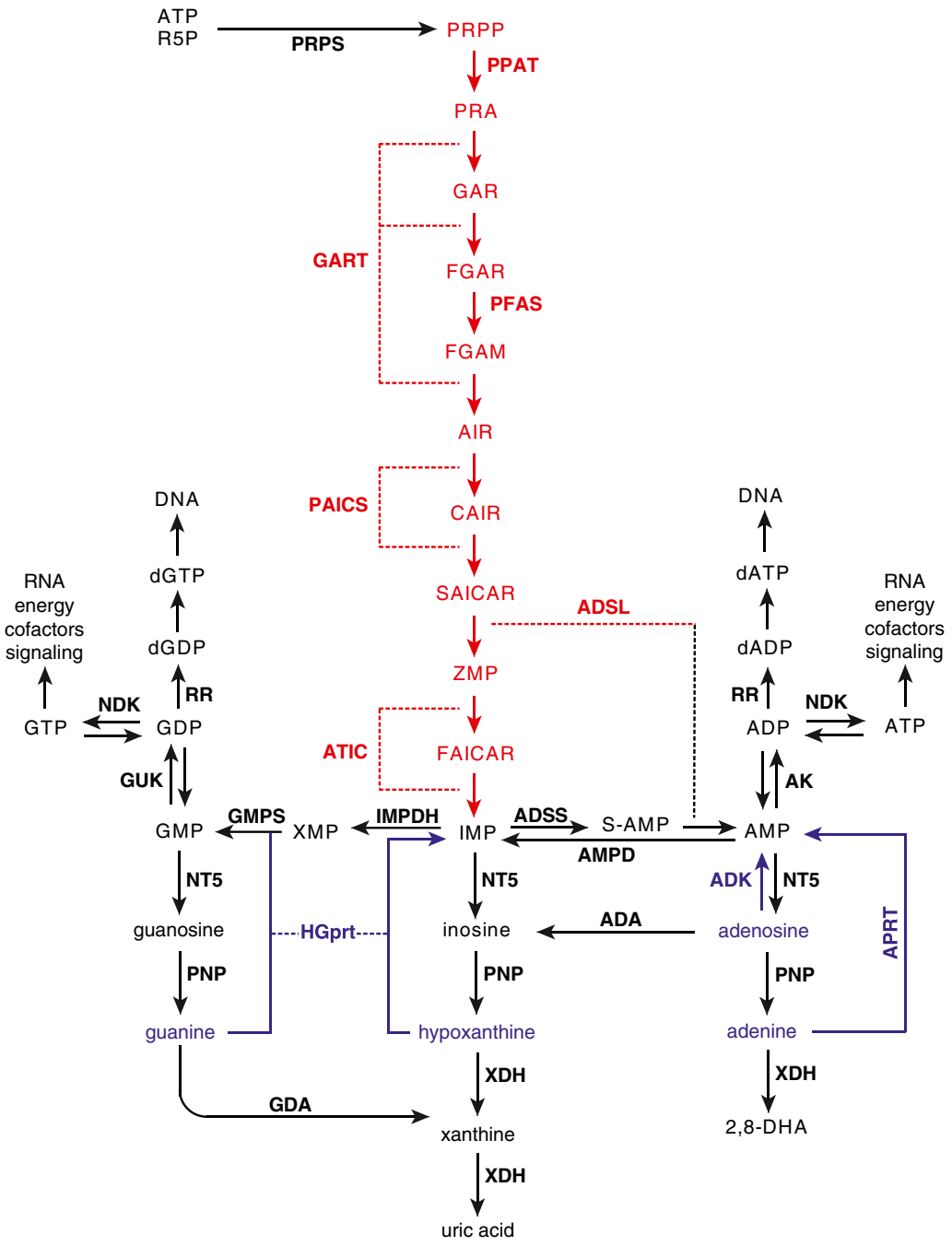

Fig. 110.2 Purine metabolism. The six enzymes and 10 reactions of de novo purine synthesis are shown in *red*. The two enzymes and three reactions of the salvage pathways are shown in *purple*. Enzyme gene names are shown in **bold font**, and metabolites are shown in regular font. For enzymes with multiple isoforms, only the core gene name is shown, without isoform numbers or letters. *Dashed lines* show enzymes with multiple functions. 2,8-DHA, Dihydroxyadenine; ADA, adenosine deaminase; ADP, adenosine diphosphate; ADK, adenosine kinase; ADSL, bifunctional adenylosuccinate lyase; ADSS, adenylosuccinate synthase; AICAR, aminoimidazole carboxamide ribotide; AMPD, adenylate deaminase; AMP, adenosine monophosphate or adenylate; APRT, adenine phosphoribosyltransferase; ATIC, bifunctional AICAR transformylase/IMP cyclohydrolase; AK, adenylate kinase; ATP adenosine triphosphate; CAIR, 5′-Phosphoribosyl-4-carboxy-5-aminoimidazole; dADP, deoxy adenosine diphosphate; dGDP, deoxy guanosine diphosphate; FAICAR, formylaminoimidazole carboxamide ribotide; GART, trifunctional phosphoribosylglycinamide formyltransferase/phosphoribosylglycinamide synthetase/phosphoribosylaminoimidazole synthetase; GDA, guanine deaminase (aka guanase or cypin); GDP, guanosine diphosphate; GMP, guanosine monophosphate or guanylate; GMPS, GMP-synthase; GTP, guanosine triphosphate; GUK, guanylate kinase; HGprt, hypoxanthine-guanine phosphoribosyltransferase; IMP, inosine monophosphate or inosinate; IMPDH, IMP-dehydrogenase; NDK, nucleoside diphosphate kinase; NT5, 5′-nucleotidase; PNP, purine nucleoside phosphorylase; PAICS, bifunctional phosphoribosylaminoimidazole carboxylase; PFAS, phosphoribosyl formylglycinamide synthase; PPAT, bifunctional phosphoribosylaminoimidazole carboxylase; PRA, 5-phosphoribosylamine; PRPP, phosphoribosylpyrophosphate; PRPS, PRPP-synthase; RR, ribonucleotide reductase; S-AMP, adenylosuccinate; SAICAR, succinylaminoimidazole carboxamide ribotide; XDH, xanthine dehydrogenase, xanthine oxidase, or xanthine oxidoreductase; XMP, xanthine monophosphate or xanthylate; ZMP, 5-aminoimidazole-4-carboxamide ribonucleotide or Z-nucleotide monophosphate. (*Copyright H.A. Jinnah.*)

expression levels of different enzymes in different tissues and sometimes tissue-specific isoforms of the same enzyme.

DISORDERS OF PURINE METABOLISM

Uric Acid

Serum uric acid levels reflect a balance among production, absorption, and excretion. Most uric acid is produced in the body as a by-product of purine metabolism (see Fig. 110.2). Some also comes from the diet, when DNA and RNA of foods are metabolized by the gut and liver. About two thirds of the body's uric acid is eliminated by the kidneys, with the remainder eliminated by the gut. Normal serum uric acid levels are age dependent. Relatively low levels in young children reach adult values by puberty. After puberty, serum uric acid is lower in females than in males.

In adults, numerous epidemiologic studies have linked abnormal serum uric acid levels with a large number of disorders. High levels of uric acid have been linked with gout, kidney disease, tophi, hypertension, diabetes mellitus, obesity, metabolic syndrome, heart disease, stroke, hemolytic anemia, Down syndrome, asthma, and others. Causal links between uric acid and disease with well-defined biologic mechanisms are well established for gout, kidney disease, and tophi. Low levels of uric acid have been linked with Parkinson disease, Alzheimer disease, multiple sclerosis, and other autoimmune disorders. For these disorders, uric acid has been proposed to have antioxidant properties, so chronically low levels may contribute to cell injury during aging.

In children, low (or even absent) levels of serum uric acid do not appear to have any detrimental clinical effects, but high levels cause problems. Uric acid is normally near its limits of solubility in the body, so even small increases risk precipitation in vulnerable regions. The joints are one of these vulnerable regions, because of the low pH of synovial fluid. Such precipitates can cause an inflammatory arthritis known as **gout**. These precipitates tend to occur in cool regions of the body, such as distal joints of the toes. The typical presentation of gout is acute inflammation of one joint, often the metatarsophalangeal joint of the large toe, although any joint can be involved. Uric acid crystals also precipitate in cool regions of the skin, forming tophi at tendon insertion points over joints or the pinna of the ear. These tophi appear as visible and palpable subcutaneous masses. Excess uric acid is concentrated by the kidneys in the renal collecting system, where high levels precipitate in two different ways. Microscopic crystals may produce an inflammatory nephropathy with painless chronic renal failure. Larger stones may cause urinary obstruction with acute renal colic, dysuria, hematuria, and recurrent urinary infections. These pathologies may also overlap in some cases.

Hyperuricemia and its pathologic consequences are common in adults, especially males. These problems often reflect an inherited predisposition for high uric acid together with lifestyle related to diet and exercise. Hyperuricemia and its consequences are very uncommon in children. Their occurrence in children often signifies an underlying disorder of purine metabolism. Uric acid is a component of many routine clinical chemistry screening panels. As a result, abnormal levels of uric acid often provide an early clue to one of these disorders (Table 110.2). Kidney stones are common in adults, but they are very uncommon in children. Nephrolithiasis and renal failure also provide an important clue to disorders of uric acid and some other disorders of purine and pyrimidine metabolism (Table 110.3). Some are rare inherited disorders affecting specific biochemical pathways; others are acquired or iatrogenic.

Individual Disorders

Individuals with **renal hypouricemia** have marked reductions in serum uric acid (<2 μg/dL or <120 μM) from birth. The disorder is autosomal recessive and caused by pathogenic variants in the *SLC22A12* or *SLC2A9* genes, which code for transporters important for renal retention of uric acid. Many affected individuals remain asymptomatic for life. Some experience renal impairments after strenuous exercise because physical activity is associated with increased purine turnover and transient elevations of serum uric acid. When a sudden bolus of uric acid after exercise is concentrated quickly in the renal collecting system, there is a transient risk for stones or "sludge" with nephropathy. This sludge reflects a somewhat viscous mixture of tiny stones and partly solubilized uric acid. Gout is uncommon in renal hypouricemia.

Familial juvenile hyperuricemic nephropathy is an autosomal dominant disorder that has been linked to pathogenic variants in *UMOD* (uromodulin), *REN* (renin), *MUC* (mucin), or *HNF*-1b (hepatocyte nuclear factor 1b). All of these genes encode proteins involved in renal handling of uric acid. Affected individuals present in childhood or adolescence with hyperuricemia, renal failure, and/or gout. Individuals with pathogenic variants in the *ABCG2* gene have reduced intestinal secretion of uric acid, along with chronic hyperuricemia and gout.

Overproduction of uric acid may also occur in inherited **disorders of carbohydrate metabolism**. They include disorders associated with pathogenic variants in *ALDOB* (aldolase B deficiency, hereditary fructose intolerance, fructosemia), *SLC37A4* (glucose-6-phosphatase deficiency, glycogen storage disease type I, or von Gierke disease), *AGL* (glycogen debranching enzyme deficiency, glycogen storage disease type III, or Cori disease), *PYGM* (muscle glycogen phosphorylase deficiency, glycogen storage disease type V, or McArdle disease), and *PFKM* (muscle phosphofructokinase deficiency, or glycogen storage disease type VII, or Tarui disease). These disorders may be associated with chronic or episodic hyperuricemia, sometimes with gout or renal impairments. Hyperuricemia is often triggered by exercise because the metabolic defect impairs energy metabolism during muscle activity and leads to catabolism of adenosine triphosphate (ATP).

Some disorders of uric acid are *acquired* rather than inherited. Because uric acid is eliminated largely by the kidneys, acute or chronic renal insufficiency may lead to hyperuricemia, which may be severe. A transient but marked elevation of serum uric acid may also occur in **tumor lysis syndrome**, where the treatment of hematologic malignancies is associated with sudden death of many cells in a short period, leading to generation of large amounts of DNA and RNA that are degraded into uric acid. The sudden bolus of uric acid is rapidly concentrated by the kidneys, leading to a risk for kidney stones, sludge, and renal failure. Relatively more modest increases in serum uric acid have been associated with other childhood disorders, including obesity, metabolic syndrome, Down syndrome, asthma, cyanotic congenital heart disease, chronic hemolytic anemia, and certain medications. Medications causing hyperuricemia include thiazide diuretics, cyclosporine, and some anticonvulsants (valproate and phenobarbital).

Treatment and Prognosis

All renal purine stones, including those made of uric acid, are radiolucent. Unless they calcify, they cannot be detected with plain films or CT, so diagnosis may require ultrasound. Stones passed in the urine may be collected and chemically analyzed. Treatment of small kidney stones may require analgesics for renal colic and/or antibiotics for associated urinary tract infection until the stones are eliminated in the urine. Larger kidney stones may obstruct the urinary collecting system and may require lithotripsy or surgical removal. Because uric acid is more soluble in an alkaline environment, alkalinization of the urine with potassium-sodium citrate or sodium bicarbonate may be useful for individuals at risk for recurrent stones.

The diagnosis of gout can be made finding typical birefringent crystals in an aspirate of joint fluid. Because purine stones are radiolucent, chronic tophaceous gout may have a punched-out appearance on plain films of the joints, where the uric acid crystals have eroded normal bone. The treatment of acute painful gout usually involves nonsteroidal antiinflammatory drugs. However, for both gout and renal stones, treatments directed at uric acid levels are essential to prevent recurrences that may lead to permanent joint damage or chronic renal insufficiency. Chronic hyperuricemia can be treated with three different strategies. One strategy is to increase renal excretion of uric acid with drugs such as probenecid, sulfinpyrazone, benzbromarone, or lesinurad. Another strategy is to use drugs that inhibit uric acid production by xanthine oxidoreductase (XOR) such as allopurinol, febuxostat, and topiroxostat. The third involves administration of recombinant uricase

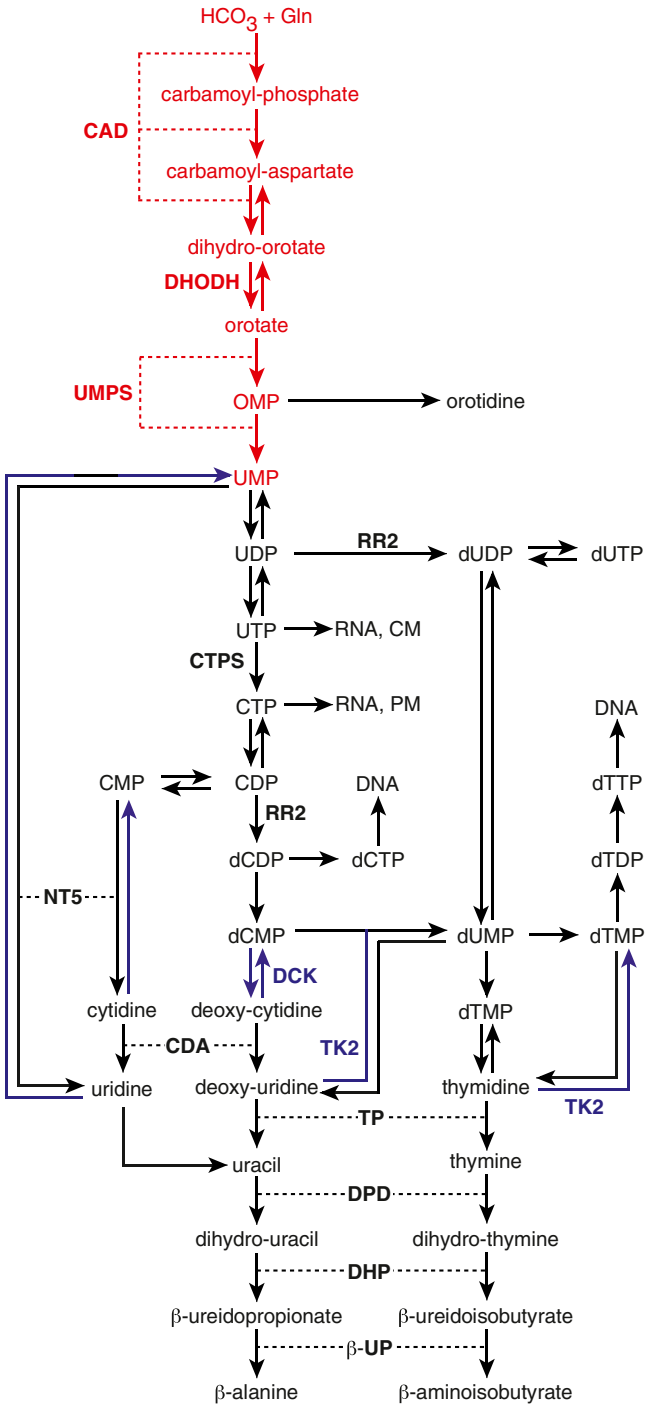

Fig. 110.3 Pyrimidine metabolism. The three enzymes and six reactions of de novo pyrimidine synthesis are shown in *red*. The enzymes of the salvage pathways are shown in *purple*. Enzyme gene names are shown in **bold font**, and metabolites are shown in regular font. For enzymes with multiple isoforms, only the core gene name is shown, without isoform numbers or letters. *Dashed lines* show enzymes with multiple functions. 5′NT, 5′-nucleotidase; β-UP, β-ureidopropionase; CAD, carbamoyl phosphate synthase; CDA, cytidine deaminase; CDP, cytidine diphosphate; CM, carbohydrate metabolism; CMP, cytidine monophosphate; CTP, cytidine triphosphate; CTPS, CTP synthase; dCDP, deoxy cytidine diphosphate; DCK; deoxycytidine kinase; dCMP, deoxycytidine monophosphate; dCTP, deoxycytidine triphosphate; DHODH, dihydro-orotate dehydrogenase; DHP, dihydropyrimidinase; DPD, dihydropyrimidine dehydrogenase; dTDP, deoxythymidine diphosphate; dTMP, deoxythymidine monophosphate; dTTP, deoxythymidine triphosphate; dUDP, deoxyuridine diphosphate; dUMP deoxyuridine monophosphate; dUTP, deoxyuridine triphosphate; gln, glutamine; OMP, orotidine monophosphate or orotic acid; PM, phospholipid metabolism; RR2, ribonucleotide reductase 2; TK2, thymidine kinase 2; TP, thymidine phosphorylase; UDP, uridine diphosphate; UMP, uridine monophosphate; UMPS, UMP synthase; UTP, uridine triphosphate. (*Copyright H.A. Jinnah.*)

(rasburicase or pegloticase), a nonhuman enzyme that can degrade uric acid.

The best choices for treatment depend on the biologic mechanisms causing excess uric acid. The many causes of hyperuricemia may be divided into two groups. The first group involves renal underexcretion, where hyperuricemia can be treated with uricosuric drugs or XOR inhibitors. The second group involves renal overload, which includes disorders where uric acid is overproduced in the body or underexcreted by the gut. Hyperuricemia associated with renal overload should be treated with XOR inhibitors, not with uricosuric drugs that further increase the burden of uric acid handled by the kidneys. Rasburicase is usually reserved for the transient marked increases in serum uric acid associated with tumor lysis syndrome. Pegloticase is usually reserved for the treatment of gout resistant to other medications.

These medications are usually combined with generous hydration to provide a constant flow of associated purine metabolites from the body. A low-purine diet is sometimes recommended as well. Some foods contain large amounts of purines, such as dried or cured meats (sardines, anchovies) and organ meats (thymus, kidney, liver). Fructose and alcohol also should be avoided because they stimulate purine turnover and result in increased uric acid production.

THE HEREDITARY XANTHINURIAS

The hereditary xanthinurias are a group of autosomal recessive disorders defined by high levels of xanthine in the urine, along with low or nondetectable serum uric acid. The estimated incidence is 0.5-1 per 100,000 live births. The metabolic abnormalities result from deficiency of XOR, the enzyme that converts hypoxanthine and xanthine into uric acid (see Fig. 110.2). XOR exists in two forms, sometimes called *xanthine oxidase* or *xanthine reductase,* both with a molybdenum-containing cofactor at the active site. These two forms are encoded by the same gene. Xanthine oxidase is derived from posttranslational modification of xanthine reductase.

Although the hereditary xanthinurias all result from XOR deficiency, distinct molecular mechanisms define three different groups. **Hereditary xanthinuria type I** is caused by pathogenic variants in the *XOR* gene. **Hereditary xanthinuria type II** is caused by pathogenic variants in the *MOCOS* gene, which encodes molybdenum cofactor sulfurase, an enzyme that sulfurates the molybdenum cofactor required for XOR as well as the enzyme aldehyde oxidase. As a result, hereditary xanthinuria type II is characterized by combined deficiency of XOR and aldehyde oxidase. The **third type of hereditary xanthinuria** is caused by defects in the synthesis of the molybdenum cofactor. Molybdenum cofactor deficiency has been linked with four genes: *MOCS1* (molybdenum cofactor synthase 1), *MOCS2* (molybdenum cofactor synthase 2), *MOCS3* (molybdenum cofactor synthase 3), and *GPHN* (gephyrin). *MOCS1* accounts for two thirds of all cases. All are required for the synthesis of the molybdenum cofactor, which is used by four enzymes including XOR, aldehyde oxidase, sulfite oxidase, and mitochondrial amidoxime reducing component. As a result, the molybdenum cofactor deficiencies are characterized by the combined defects of all four enzymes.

Clinical Features

The clinical features of hereditary xanthinuria type I caused by isolated XOR deficiency may be benign. Many individuals are asymptomatic. The increased excretion of xanthine leads to kidney stones in approximately one third of individuals. These stones may develop any time from early childhood through later adulthood. Clinical signs may be acute with dysuria, hematuria, renal colic, or a history of recurrent urinary tract infections. Alternatively, affected individuals may present with acute or chronic renal failure. Xanthine stones, like other purine stones, are radiolucent; they are detectable with ultrasound but may be missed on abdominal plain films or CT unless they calcify. Some individuals may develop myopathy after strenuous exercise with xanthine crystals in muscle because of the rapid turnover of purines associated with exercise.

The clinical features of combined deficiency of XOR and aldehyde oxidase in hereditary xanthinuria type II are similar to those of type I. There appears to be no obvious clinical impact of the added deficiency of aldehyde oxidase.

Table 110.2 Disorders Influencing Uric Acid

DISORDER	GENE SYMBOL	SERUM URIC ACID	URINARY URIC ACID
Hereditary xanthinurias			
Type I	*XOR*	↓↓	↓↓
Type II	*MOCOS*		
Molybdenum cofactor deficiencies	*MOCS1-3, GPHN*		
HPRT1-associated disorders	*HPRT1*	↑	↑↑
PRPP dysregulation disorders	*PRPS1*	↑	↑
Excretion disorders			
Renal hypouricemia	*SLC22A12, SLC2A9*	↓↓	↑↑
Reduced gut excretion	*ABCG2*	↑	↑
Carbohydrate disorders			
Fructosemia	*ALDOB*	↑ (chronic or exercise-induced)	↑ (chronic or exercise-induced)
Glucose-6-phosphatase deficiency	*SLC37AY*		
Glycogen debranching deficiency	*AGL*		
Glycogen phosphatase deficiency	*PYGM*		
Phosphofructokinase deficiency	*PFKM*		
Renal insufficiency	NA	↑	↓
Tumor lysis syndrome	NA	↑↑	↑↑

NA, not applicable. Copyright H.A. Jinnah.

Table 110.3 Renal Stones in Disorders of Purines and Pyrimidines

DISORDER	GENE SYMBOL	STONES
APRT deficiency	*APRT*	Dihydroxy-adenine
Familial juvenile hyper-uricemic nephropathy	*UMOD, REN, MUC,* or *HNF-1*b	Uric acid
Hereditary orotic aciduria	*UMPS*	Orotic acid
Hereditary xanthinurias		
Type 1	*XOR*	Xanthine
Type 2	*MOCOS*	Xanthine
Molybdenum cofactor deficiencies	*MOCS1-3, GPHN*	Xanthine
HPRT1-associated disorders	*HPRT1*	Uric acid
PRPP synthetase hyperactivity	*PRPS1*	Uric acid
Tumor lysis syndrome	Not applicable	Uric acid

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The clinical features of the **molybdenum cofactor deficiencies** are more severe. Individuals often present with failure to thrive during infancy, difficulties feeding, intractable seizures, psychomotor delay, and microcephaly. Brain MRI may show diffuse atrophy or cerebellar hypoplasia, multicystic encephalomalacia, and signal abnormalities of the globus pallidus. Eye findings may include ectopic lens, spherophakia, and nystagmus. Most die in early childhood.

Because uric acid is included in many routine blood chemistry screening panels, the diagnosis of the hereditary xanthinurias often starts with serendipitous identification of low or absent uric acid. Instead, urinary xanthine and hypoxanthine are elevated. However, xanthine is elevated more than hypoxanthine, because the latter can be recycled by HGprt or cleared by the kidneys. In individuals with hereditary xanthinuria type II with combined deficiency of XOR and aldehyde oxidase, urinary methyl-2-pyridone-carboxamide also is low or absent. The characteristic pattern observed among individuals with the molybdenum cofactor deficiencies includes low uric acid in serum and urine along with high urinary xanthine, hypoxanthine, sulfite, and S-sulfocysteine. Enzymatic tests for XOR are feasible but not commonly used for clinical diagnosis. They require biopsy of the liver or small bowel where the amounts of this enzyme reach high enough levels for measurement. A convenient test for aldehyde oxidase involves assessing its ability to oxidize a test dose of allopurinol to oxypurinol. Sulfite oxidase and molybdenum cofactor can be measured in liver or fibroblasts. A molecular diagnosis can be made by finding pathogenic variants in one of the relevant genes.

Treatment and Prognosis

The treatment of all hereditary xanthinurias involves generous hydration to constantly flush xanthine from the renal collecting system to avoid stone formation. Hydration is sometimes combined with a low-purine diet.

Aside from palliative care, minimal success has been achieved with numerous approaches to the treatment of the molybdenum cofactor deficiencies. However, cyclic pyranopterin monophosphate (fosdenopterin) can have a life-changing impact on the normally serious consequences of molybdenum cofactor deficiency associated with *MOCS1*. Treatment has been reported to normalize metabolic measures and allow near-normal development among individuals who start it at a very early age before there is significant brain damage. It is approved for use in individuals with *MOCS1* defects by the U.S. Food and Drug Administration (FDA).

HPRT1-ASSOCIATED DISORDERS

Disorders associated with the *HPRT1* gene are rare, with an overall prevalence of approximately three cases per million. Inheritance is X-linked and recessive, although sporadic cases are not uncommon. The gene encodes hypoxanthine-guanine phosphoribosyl transferase (HGprt), an enzyme responsible for two different reactions (see Fig. 110.2). In one reaction, the cosubstrate phosphoribosyl pyrophosphate (PRPP) is combined with the purine base hypoxanthine to produce IMP. In the other reaction, PRPP is combined with guanine to produce GMP. IMP and GMP are then recirculated into the nucleotide pools. When HGprt activity is impaired, its substrates accumulate. Accumulation of PRPP leads to excessive drive of purine synthesis, because PRPP is also used in the first and rate-limiting reaction of the de novo synthetic pathway. The substrates hypoxanthine and guanine also accumulate, and they are ultimately catabolized to uric acid. The combination of accelerated purine synthesis and increased catabolism of hypoxanthine and guanine results in a marked overproduction of uric acid. This overproduction of uric acid is responsible for several clinical features of *HPRT1*-associated disorders, including hyperuricemia, nephrolithiasis, gout, and tophi.

Overproduction of uric acid *is not* responsible for all the clinical features of HGprt deficiency. Reduced HGprt activity also leads to impaired recycling of hypoxanthine and guanine, with purine wasting. This loss of purines may lead to purine deficiency, especially in tissues where the synthetic pathway cannot compensate for purines lost due to the failure of recycling. Erythrocytes lack a fully operational synthetic pathway, so they are dependent on recycling purines taken up from the blood to maintain purines. These observations may account for *macrocytic anemia,* which is common in *HPRT1*-associated disorders.

A similar mechanism may occur in the brain, where purine recycling is important because purine synthesis is relatively low. In individuals

with HGprt deficiency, overt structural malformations are not usually found when performing routine clinical CT or MRI of the brain. Such studies are usually normal or they show only mild diffuse atrophy. However, advanced imaging methods have revealed several abnormalities. Quantitative MRI-based, voxel-based morphometry has revealed subjects with *HPRT1*-associated disorders to have volume loss in several brain regions. Similar studies, along with diffusion tensor imaging MRI, have revealed diffuse loss of white matter. The basal ganglia appear to be more affected than most other regions. In fact, PET scans have revealed a significant reduction in markers associated with basal ganglia dopamine systems.

Autopsy studies of the brains of individuals with *HPRT1*-associated disorders do not show signs of maldevelopment or neurodegeneration. Dopamine neurons are present, but they do not express normal levels of dopamine-related markers. Dysfunction of the basal ganglia is thought to be responsible for several of the neurologic features of *HPRT1*-associated disorders, including dystonia and self-injurious behavior. Dysfunction of corticospinal pathways may be responsible for cognitive impairment, spasticity, and seizures.

Clinical Features

Pathologic genetic variants in *HPRT1* are associated with a spectrum of clinical phenotypes (Fig. 110.4). Most cases are males, although rare females with defects in both *HPRT1* alleles have been described. More than 600 different gene variants are known. A complete loss of HGprt function is associated with most of these variants, such as deletions, insertions, nonsense substitutions, and some single amino acid substitutions. A partial loss of HGprt function is associated with other variants, usually conservative single amino acid substitutions and some splice variants.

The severity of the clinical phenotypes is related to the degree of associated enzyme impairment. The spectrum of clinical phenotypes is usually subdivided into three overlapping groups. *HPRT1* variants associated with a mean value of 12% of normal HGprt activity are associated with the mildest clinical phenotype. This phenotype is known as **HGprt-related hyperuricemia**. These individuals experience overproduction of uric acid, along with associated problems of hyperuricemia, nephrolithiasis, gout, and tophi. These individuals do not have overt neurologic or behavioral problems, although mild clumsiness or mild cognitive impairments may be disclosed with careful testing. The age at presentation ranges from infancy to adulthood, usually with renal problems or gout.

HPRT1 variants associated with a mean of 7% of normal HGprt activity are associated with an intermediate phenotype with overproduction of uric acid along with clinically overt neurologic impairments. This phenotype is known as **HGprt-related neurologic dysfunction**. Motor impairments vary widely and may range from minor clumsiness to a phenotype that resembles severe dyskinetic cerebral palsy. Cognitive deficits may range from attention-deficit disorder to moderate intellectual disability. These individuals typically present with delayed motor development, although some present with renal problems or gout.

HPRT1 variants associated with a mean value of 1% normal HGprt activity are associated with the most severe phenotype, known as **Lesch-Nyhan disease**. These individuals have overproduction of uric acid, disabling motor impairments, moderate intellectual disability, and an unusual behavioral syndrome. The motor problems begin with delayed development in infancy and evolve similar to severe dyskinetic cerebral palsy. Epilepsy is common. The most characteristic aspect of the behavioral syndrome is severe recurrent self-injury, with self-biting, self-hitting, recurrent scratching or poking, and others. Self-injury typically begins between 2 and 4 years of age but may be delayed until the teenage years. Other difficult behaviors are also common, such as impaired attention and impulsivity. Difficult behaviors may be also directed toward others. They include use of language that is foul, sexually inappropriate, or racially charged. Also frequent are hitting, grabbing, or spitting.

The diagnosis of any *HPRT1*-associated disorder should be suspected in a child or young adult with evidence for overproduction of uric acid such as hyperuricemia, uric acid nephrolithiasis, or gout. Uric acid (pink) crystals in diapers during infancy are frequently the first clue. Suspicion for an *HPRT1*-associated disorder increases when there is evidence for overproduction of uric acid along with a history of delayed motor development. The emergence of self-injurious behavior between 2 and 4 years of age often provides a more specific clue for Lesch-Nyhan disease. A definitive diagnosis requires demonstration of a pathogenic variant in the *HPRT1* gene and/or enzymatic evidence for reduced HGprt function.

Treatment and Prognosis

For all *HPRT1*-associated disorders, excessive production of uric acid must be treated to avoid renal complications and gouty arthritis. Treatment requires a lifelong combination of an inhibitor of XOR to reduce production of uric acid and generous hydration. The commonly used XOR inhibitor is allopurinol, although others can also be used. Doses are titrated to maintain serum uric acid within normal limits. Stones may continue to form despite treatment, and lithotripsy or surgical removal is sometimes required. High doses of XOR inhibitors that reduce serum uric acid to very low levels are discouraged, because this approach increases the risk of stones composed of the precursors xanthine and hypoxanthine. Some specialists advocate alkalinization of the urine to promote solubility of uric acid or a low-purine diet to reduce intake of purines that are metabolized to uric acid.

The motor disorder is dominated by dystonia, sometimes with spasticity. The increased muscle tone associated with these problems is most often treated with muscle relaxants such as benzodiazepines or baclofen. Anticholinergics and dopamine-related drugs are not generally successful. Several case reports describe great success with deep brain stimulation surgery, but the rate of surgical complications is unusually high, and the overall risk-benefit ratio does not favor universal recommendation of this approach.

The behavioral problems require a combination of physical protective devices, specific behavioral modification techniques, and sometimes pharmacotherapy. Most individuals with Lesch-Nyhan disease require regular restraints of the hands and arms to prevent self-hitting and biting of the fingers. Approximately half of all individuals require tooth removal to prevent biting of the lips and tongue. A custom-designed wheelchair with dangerous regions shielded is often needed. The most useful behavioral techniques involve extinction (ignoring) and redirection (distraction). Methods that use positive reinforcement are useful, but methods that use negative reinforcement amplify negative behaviors.

Individuals with the mildest clinical phenotypes may have a normal life span, provided uric acid is well-managed. Those with more severe phenotypes have a shorter life span, with death occurring in the teens through the fifth decade. Most frequent causes of death include aspiration pneumonia or complications from renal dysfunction. Cases of sudden unexplained death have also been reported.

APRT-ASSOCIATED DISORDERS

Disorders associated with the *APRT* gene are rare, with fewer than 1,000 individuals reported. More than 200 pathogenic variants have been described. Inheritance is autosomal recessive. The disorder has been reported worldwide, but there are clusters of individuals with the same genetic variants originating in Japan, France, and Iceland.

The *APRT* gene encodes adenine phosphoribosyl transferase (APRT), an enzyme that is responsible for catalyzing a reaction in which PRPP is combined with the purine base adenine to produce AMP (see Fig. 110.2). Deficiency of APRT leads to accumulation of adenine, which is metabolized by XOR into 2,8-dihydroxyadenine (DHA). DHA does not accumulate in serum because it is rapidly cleared by the kidneys. Because solubility of DHA is poor, it precipitates in the urinary collecting system as either small crystals or larger stones. Small crystals cause chronic renal insufficiency because they provoke tubulointerstitial nephritis, inflammation, and fibrosis. Larger stones may cause acute obstructive renal failure.

Clinical Features

Clinical manifestations are attributable to the kidneys, and they may develop in a wide age range from infancy through adulthood.

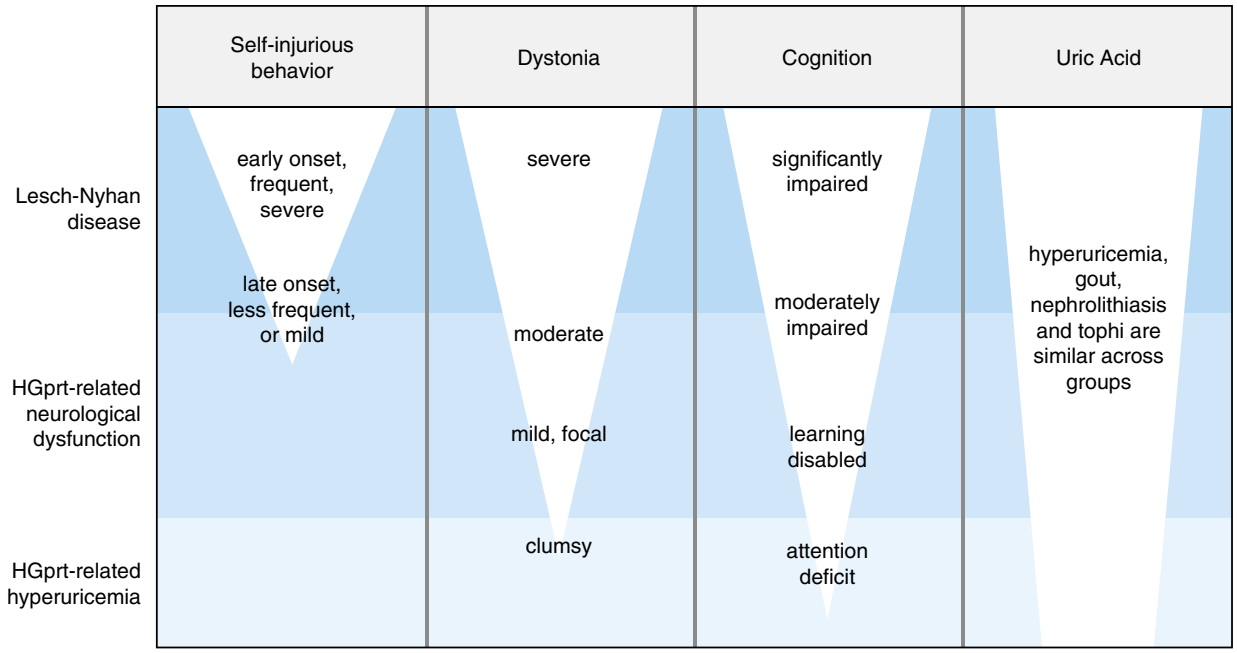

Fig. 110.4 *HPRT1*-associated disorders. This schematic provides a graphical representation of the overlapping spectrum of *HPRT1*-associated disorders. The three disorders are listed on the left side of the panel. The most common clinical features are shown in the top row. The *white triangles* depict the severity of individual clinical features for each of the disorders. For example, problems related to overproduction of uric acid occur in all phenotypes, but self-injurious behavior is limited to the most severe phenotype. Dystonia and cognition both show a wide range of severity across all groups, but severity correlates with overall phenotypic subgroup. This schematic provides only a general guide, as some exceptions may occur. (*Copyright H.A. Jinnah.*)

Approximately half of individuals with APRT deficiency have no symptoms, or symptoms may develop only in old age. The initial presentations fall into two groups which may overlap: nephrolithiasis and DHA nephropathy. Individuals with nephrolithiasis may present acutely with dysuria, hematuria, renal colic, or recurrent urinary tract infections. They may describe accompanying crystals, larger stones, or gravel in the urine. The precipitates are often reddish-brown in color and easily crushed. Alternatively, DHA nephropathy may result in a more slowly evolving renal insufficiency, with approximately 15% first presenting with end-stage renal failure.

The diagnosis should be suspected when there is evidence for crystalluria or nephrolithiasis, especially in a child. DHA stones, like other purine stones, are radiolucent. Unless they calcify, they may be detected via ultrasound but not with abdominal plain films or CT. The diagnosis may also be suspected in individuals with unexplained renal failure who undergo renal biopsy revealing a crystallopathy with tubulointerstitial nephritis, inflammation, and/or fibrosis. APRT deficiency must be distinguished from other disorders that involve nephrolithiasis and/or renal failure.

Commonly used clinical biochemical methods may not be able to distinguish DHA stones from other purine stones, but more specific methods have been developed at some specialized centers. DHA crystals in the urine can also be identified by light and polarizing microscopy, along with infrared spectrophotometry. Crystals in renal biopsy specimens can be identified by polarizing microscopy with infrared microscopy. Definitive diagnosis can also be achieved by measuring APRT enzyme activity in erythrocytes or by documenting pathogenic variants in the *APRT* gene.

Treatment and Prognosis

Treatment requires an inhibitor of XOR to reduce production of DHA. The commonly used XOR inhibitors include allopurinol or febuxostat, and they must be continued lifelong. Generous hydration is useful to flush purine metabolites through the renal collecting system. A low-purine diet is sometimes recommended. Large stones may require lithotripsy or surgical removal. Individuals who are diagnosed early in life and adhere to therapy may have a normal life span. Individuals who are diagnosed late may have chronic renal insufficiency or even renal failure that requires dialysis or kidney transplantation.

DISORDERS ASSOCIATED WITH AMP-DEAMINASE

The enzyme AMP-deaminase catalyzes the deamination of AMP to IMP with simultaneous production of NH_3. The IMP can then be recirculated either to AMP or GMP (see Fig. 110.2). The enzyme is encoded by three homologous genes. *AMPD1* is expressed predominantly in muscle, *AMPD2* is broadly expressed with high levels in the brain, and *AMPD3* is broadly expressed at high levels in bone marrow cells. Each gene has been associated with genetic variants producing null enzyme activity.

Clinical Features

For *AMPD1,* approximately 20% of White and Black individuals have a c.34C>T variant producing an early termination codon (p.Q12>X) in exon 2. In these populations, 1–2% are homozygotes with null enzyme activity in muscle. Most homozygotes have no symptoms, but **myopathic features** have been reported for some. These features include exercise intolerance with fatigue or myalgia, sometimes with muscle cramping or increased serum creatine kinase. Symptoms may begin at any age from early childhood to later adulthood. Histochemical stains for AMP-deaminase are negative in muscle biopsy specimens, but frank myopathic features are usually lacking. Because of the large numbers of nonsymptomatic individuals, the role of the genetic variant in causing symptoms is not clear. The variant may be benign, or it may combine with other genes or factors to cause symptoms. A diagnosis can be made via the forearm exercise test with measures of lactate and NH_3 production, histochemical staining of the enzyme in a muscle biopsy, or finding a variant in the *AMPD1* gene.

For *AMPD2,* pathogenic variants have been linked with **pontocerebellar hypoplasia** (PCH9). This neurologic disorder is rare, with fewer than 50 cases reported worldwide. Pontocerebellar hypoplasia is a group of disorders described by dysgenesis of the pons and cerebellum. Individuals with the PCH9 subtype also have dysgenesis of the

corpus callosum and microcephaly. Clinical symptoms include severe psychomotor delay, central visual impairment, and seizures.

ADSL-ASSOCIATED DISORDERS

Disorders associated with the *ADSL* gene are rare, with fewer than 100 individuals reported. The disorder is autosomal recessive. Pathogenic gene variants with complete loss of enzyme activity are likely incompatible with life. The pathogenic variants associated with disease produce a partial loss of enzyme function. The enzyme is bifunctional, and it mediates two distinct reactions in purine synthesis (see Fig. 110.2). Reduced enzyme function leads to accumulation of succinylpurines (succinyl-aminoimidazole carboxamide riboside and succinyl-adenosine), which are eliminated in the urine. Historically, this disorder was therefore called succinyl-purinuria. The clinical manifestations are thought to result predominantly from pathologic effects of succinyl purines in the brain. Brain MRI reveals varied findings from nearly normal to global or regional atrophy with delayed myelination.

Clinical Features

The clinical manifestations are often described in three distinct groups, but the groups overlap with a graded spectrum of severity. The most severely affected group is described as a **fatal neonatal encephalopathy**. These individuals have a severe paucity of spontaneous movement from birth, intractable seizures, and respiratory failure. They die within the first weeks of birth. Individuals in the **intermediate group** (type I) present with hypotonia and motor delay. They survive for longer periods, with moderate to severe psychomotor delay and seizures. Abnormal behaviors have been described as autistic with poor eye contact, repetitive stereotyped movements or sounds, and frequent agitation. The least severely affected group is referred to as **type II**. They present within the first years of life with evidence for psychomotor delay, sometimes with seizures and behavioral problems. The diagnosis is made by finding elevated succinyl purines in urine or cerebrospinal fluid (CSF) or by finding pathogenic variants in the *ADSL* gene.

Treatment and Prognosis

Treatment is palliative. Seizures may be refractory to conventional anticonvulsants, but some success has been reported with the ketogenic diet.

OTHER INHERITED PURINE DISORDERS

Adenosine Deaminase

Deficiency of adenosine deaminase results in **severe combined immunodeficiency syndrome (SCID)**, with defects in both humoral (B cells) and cellular (T cells) immunity. Individuals with severe SCID present shortly after birth with life-threatening infections. Individuals with milder disease may present at 2-4 years of age, or even older (see Chapter 165.1). In contrast with adenosine deaminase deficiency, superactivity of the same enzyme causes hemolytic anemia.

Adenosine kinase. Adenosine kinase is sometimes considered among the purine salvage enzymes because it recycles adenosine into adenosine monophosphate (AMP). Pathogenic variants of the associated *ADK* gene in multiple cases from independent families have been linked with hypermethioninemia, psychomotor delay, seizures, and multiple dysmorphic features. The disorder is autosomal recessive.

Adenylate kinase. There are four isoforms of adenylate kinase encoded by genes *AK1-4*. The enzyme phosphorylates AMP to ADP (see Fig. 110.2). The *AK1* gene is highly expressed in erythrocytes. Pathogenic variants in *AK1* in multiple cases from independent families have been linked with nonspherocytic hemolytic anemia, sometimes with cognitive disability.

The *AK2* gene is more broadly expressed but localizes to mitochondria. Pathogenic variants in *AK2* in multiple cases from independent families have been linked with reticular dysgenesis, a type of SCID, often with sensorineural hearing loss. Disorders associated with *AK1* and *AK2* are autosomal recessive.

Adenylosuccinate synthase. The *ADSS* gene encodes adenylosuccinate synthase, an enzyme involved in the conversion of IMP to AMP (see Fig. 110.2). There are multiple isoforms, and pathogenic variants in *ADSSL1* have been linked with a form of **myopathy**, **MPD5**. The disorder is autosomal recessive, usually begins in adolescence, and is slowly progressive. One study of affected individuals indicated that distal leg weakness was the most common feature, often with weakness of the hands. Approximately one third had weakness of the face or jaw muscles leading to difficulty with mastication. Some had left ventricular hypertrophy. Involvement of the diaphragm may lead to respiratory insufficiency in later stages of the illness.

ATIC-associated disorders. Disorders associated with the *ATIC* gene are very rare. Only four cases from three families have been reported. The disorder is autosomal recessive. The enzyme is bifunctional and mediates two distinct reactions (see Fig. 110.2). Complete loss of the enzyme is probably incompatible with life. Reduced enzyme function leads to accumulation of ZMP along with its phosphorylated derivatives. ZMP and its derivatives can be detected with the Bratton-Marshall test, a routinely used neonatal screen in some countries. All reported cases had prenatal and postnatal growth restriction, severe to profound psychomotor delay, chorioretinal atrophy with severe visual impairment, and dysmorphic facial features. Some had epilepsy. Treatment is palliative.

Deoxyguanosine kinase. The *DGK* gene encodes deoxyguanosine kinase, an enzyme that phosphorylates deoxypurine nucleosides into their respective deoxynucleotides for DNA synthesis (see Fig. 110.2). The gene is encoded by the nucleus, but the enzyme functions in mitochondria. Gene defects lead to an impairment of mitochondrial DNA synthesis, so the disorder is classified among the mitochondrial DNA depletion disorders. The disorder is rare and inherited in an autosomal recessive fashion. The clinical features reflect an early-onset multiorgan disorder, most notably liver failure, and psychomotor delay.

IMPDH-associated disorders. IMP-dehydrogenase is an enzyme that stands at a branchpoint in purine metabolism, directing the synthesis of purines toward guanine-based nucleotides rather than adenine-based nucleotides (see Fig. 110.2). This enzyme has two isoforms encoded by distinct but homologous genes. The *IMPDH1* gene is expressed primarily in the retina, whereas the *IMPDH2* gene is expressed in most other tissues. Disorders associated with these genes are rare, with only a handful of families reported.

Pathogenic variants in *IMPDH1* have been linked with **autosomal dominant retinopathy**. Affected individuals may have severe congenital retinal dysfunction with vision loss and pendular nystagmus **(Leber congenital amaurosis, LCA11)**. Others have childhood-onset retinitis pigmentosa with progressive visual impairment **(retinitis pigmentosa, RP10)**. There are no known treatments for either *IMPDH*-associated disorder.

Pathogenic variants in *IMPDH2* have been linked with an autosomal dominant neurologic disorder. Affected individuals may present with developmental delay, infantile dystonia, and seizures. Others present with a milder phenotype of dystonia that emerges in childhood or adolescence. There are no known treatments.

Phosphoribosylaminoimidazole carboxylase. The *PAICS* gene encodes a bifunctional enzyme that mediates two steps in the synthesis of purines (see Fig. 110.2). Pathogenic variants associated with *PAICS* are very rare, with only two individuals from a single family identified by exome sequencing. Both had multiple craniocervical malformations, and one had additional limb malformations. Both died within a few days of birth from respiratory failure.

Purine nucleoside phosphorylase. Deficiency of purine nucleoside phosphorylase results in selective dysfunction of T cells with susceptibility to viral illnesses. Two thirds have neurologic abnormalities, and one third have anemia. This disorder is covered in more detail in Chapter 165.2.

DISORDERS OF PYRIMIDINE METABOLISM

CAD Deficiency

The *CAD* gene encodes a trifunctional enzyme that mediates three of the initial six steps in pyrimidine synthesis (see Fig. 110.3). The disorder takes its name from the three relevant enzymatic activities, which include carbamoyl-phosphate synthetase, aspartate transcarbamylase,

and dihydroorotase. The associated clinical disorder is autosomal recessive.

Clinical Features

Fewer than 10 cases have been reported. Most presented in infancy with developmental delay, anisocytosis, and poikilocytosis. Most had medication-refractory seizures by 2 years of age, although epilepsy may start later. Many cases had global or cerebellar brain atrophy on MRI. The disorder cannot be identified by any of the metabolic tests routinely used for clinical diagnosis, but the activity of the enzyme can be measured. The disorder can also be identified by finding pathogenic variants in the *CAD* gene.

Treatment and Prognosis

Although it is quite rare, early diagnosis is important because treatment with uridine produces remarkably positive outcomes. Most of the subjects who were treated experienced a significant reduction in seizures, correction of hematologic abnormalities, and partial reversal of neurodevelopmental impairments. The disorder is therefore sometimes called **uridine-responsive epileptic encephalopathy**. It is likely that the degree of improvement depends on instituting treatments before significant brain damage occurs.

Dihydroorotate Dehydrogenase Deficiency

The *DHODH* gene encodes an enzyme that mediates the fourth of six steps in pyrimidine synthesis. The enzyme is localized in mitochondria, where electrons from the dehydrogenase are transferred to ubiquinone in the electron transport chain. A complete loss of the enzyme is not compatible with life, so all affected cases have had some residual enzyme function. The disorder is autosomal recessive.

Clinical Features

Fewer than 100 cases have been reported. Affected individuals have **Miller syndrome**, one of the acrofacial dysostosis syndromes where dysmorphic facial features are combined with abnormalities of the distal limbs. The facial anomalies include malar hypoplasia (underdeveloped cheekbones), micrognathia (small jaw), orofacial clefts (incomplete fusion of the roof of the mouth or lips), hypoplastic lower eyelids, and cup-shaped ears. The limb anomalies include abnormalities of the fifth and/or fourth fingers and toes (webbing, fusion, or hypoplasia), short forearms (ulnar hypoplasia sometimes with fusions to the radius), and hypoplasia of the fibula. Some cases also have other structural abnormalities such as pectus excavatum, rib defects, accessory nipples, or involvement of internal organs.

Treatment and Prognosis

There are no specific treatments for this disorder.

Hereditary Orotic Aciduria

The *UMPS* gene encodes a bifunctional enzyme that mediates two of the last six steps in pyrimidine synthesis (see Fig. 110.3). The relevant enzymatic activities are carried out by a single protein and include orotate phosphoribosyltransferase and orotidine 5′-monophosphate decarboxylase. These activities are sometimes combined under the term *UMP synthase,* and the disorder is sometimes known as *UMP synthase deficiency*. Affected individuals cannot convert orotic acid into UMP, so they excrete large amounts of orotic acid in the urine. Levels may be so high that orotic acid crystals may precipitate in the renal collecting system. Inheritance is autosomal recessive.

Clinical Features

The disorder is rare, with fewer than 50 reported cases. Most present in the first few weeks or months of age with failure to thrive and developmental delay. The majority have megaloblastic anemia, hypochromia, anisocytosis, and poikilocytosis. A few have had strabismus, congenital malformations, or seizures. Heterozygous carriers may excrete high levels of orotic acid without any additional symptoms.

The diagnosis is often first suspected by finding high levels of orotic acid in the urine. UMP synthase deficiency is not the only condition associated with high urinary orotic acid. Other disorders include urea cycle defects (e.g., ornithine transcarbamylase deficiency), some mitochondrial disorders, Reye syndrome, or treatment with medications (allopurinol and 6-azauridine). A definitive diagnosis of hereditary orotic aciduria comes from genetic testing that reveals pathogenic variants in the *UMPS* gene, and the enzyme can be measured in blood cells or fibroblasts.

Treatment and Prognosis

Although rare, early diagnosis is important because it is treatable with uridine. In general, supplements with uridine can reverse hematologic abnormalities and stimulate normal growth and development. Long-term treatments are associated with good results. Uridine triacetate is approved for treatment by the FDA.

Thymidine Phosphorylase Deficiency

The *TYMP* gene encodes thymidine phosphorylase, an enzyme responsible for converting thymidine to thymine (see Fig. 110.3). Deficiency of the enzyme results in marked elevations in blood thymidine. The disorder is autosomal recessive. Accumulation of phosphorylated derivatives of thymidine and deoxyuridine result in abnormal DNA replication in mitochondria and depletion of mitochondrial DNA. The enzyme has had two additional names used in the literature prior to its isolation and molecular identification. It is known as *platelet-derived endothelial cell growth factor* because of its angiogenic properties. It also is known as *gliostatin* because of a suppressive effect on the growth of glia.

Clinical Features

The disorder is rare and responsible for **mitochondrial neurogastrointestinal encephalomyopathy (MNGIE)**. Symptoms begin in adolescents or young adults. Initial symptoms are often attributable to the gastrointestinal system and include diarrhea, vomiting, malabsorption, weight loss, and episodes of pseudoobstruction. Neurologic features include progressive external ophthalmoparesis, ptosis, myopathy, neuropathy, and hearing loss.

Treatment and Prognosis

There is no specific treatment for the early-onset severe phenotype. Treatment with 5-fluorouracil and related drugs is contraindicated.

DIHYDROPYRIMIDINE DEHYDROGENASE DEFICIENCY

The *DPD* gene encodes dihydropyrimidine dehydrogenase, the first enzyme involved in the catabolism of thymine and uracil (see Fig. 110.3). Genetic variants are common. Screening studies have suggested a prevalence of 3–5% for partial deficiency and 0.1–0.5% for severe deficiency. Severe enzyme deficiency is inherited in an autosomal recessive manner, and it is associated with marked elevations of thymine and uracil in urine, blood, and CSF. The associated disorder is therefore sometimes called **thiamine-uraciluria**.

Clinical Features

The clinical manifestations are quite varied, even among individuals who carry the same genetic variant. Some individuals with severe enzyme deficiency have no symptoms, and they are identified in adulthood because of severe toxicity after treatment with 5-flurouracil or related drugs. Others are identified in childhood, although fewer than 50 cases have been reported. Approximately half of these cases have had psychomotor delay, often with seizures. A few have presented with growth restriction, microcephaly, ocular abnormalities, or autistic behaviors.

Treatment and Prognosis

There is no specific treatment for the early-onset severe phenotype. For individuals with partial deficiency, treatment with 5-fluorouracil and related drugs is contraindicated.

Dihydropyrimidinase Deficiency

The *DPYS* gene encodes dihydropyrimidinase, the second enzyme involved in the catabolism of thymine and uracil (see Fig. 110.3).

Approximately 9% of individuals of European ancestry have genetic variants that substantially reduce enzyme activity, and 0.5% have almost no activity. Associated disorders are autosomal recessive. Severe enzyme deficiency is associated with marked elevations of dihydrothymine and dihydrouracil and in urine, blood, and CSF. The associated disorder is therefore sometimes called **dihydropyrimidinuria**.

Clinical Features

Genetic variants in *DPYS* are associated with very different clinical manifestations, even among individuals who carry the same genetic variant. The severe form of the disorder is rare, with fewer than 50 reported individuals. Some individuals with severe enzyme deficiency have no symptoms. Others are identified in childhood, with approximately half having psychomotor delay and a third having seizures. Gastrointestinal problems are common and include feeding difficulties with recurrent vomiting, malabsorption, and gastroesophageal reflux. A few have presented with growth restriction, microcephaly, or autistic behaviors.

Treatment and Prognosis

There is no specific treatment for the early-onset severe phenotype. Testing for common genetic variants before the use of 5-fluorouracil and related drugs has been recommended.

β-Ureidopropionase Deficiency

The *UPB1* gene encodes β-ureidopropionase, also known as *β-alanine synthase*. It is responsible for the last step of pyrimidine catabolism (see Fig. 110.3). Deficiency of the enzyme leads to accumulation of *N*-carbamoyl-β-alanine and *N*-carbamyl-β-aminoisobutyric acid in urine, blood, and CSF. As a result, the disorder is sometimes called ***N*-carbamoyl-β-amino aciduria**. The mechanism by which the enzyme defect leads to clinical disease is not well known.

Clinical Features

The disorder is autosomal recessive and rare, with fewer than 50 reported individuals. Affected individuals have psychomotor delay, seizures, microcephaly, and autistic behaviors. However, enzyme screening has revealed some individuals with no apparent symptoms. Diagnosis can be made by measuring accumulation of the associated metabolites, enzyme testing from liver biopsy, or finding pathogenic variants in the gene.

Treatment and Prognosis

There is no specific treatment for this disorder.

DISORDERS ASSOCIATED WITH THYMIDINE KINASE

Dividing cells rely on de novo synthesis of pyrimidines to make most of their nucleotides, but these pathways are downregulated in postmitotic cells, which primarily maintain pyrimidine nucleotides instead via salvage (see Fig. 110.3). Salvage enzymes include cytosolic thymidine kinase (*TK1* gene) and mitochondrial thymidine kinase (*TK2* gene). The *TK1* gene is often overexpressed in rapidly dividing cells and used as a marker for early detection or recurrence of cancer.

Pathogenic variants in the *TK2* gene impair the phosphorylation of thymidine and deoxycytosine, leading to reductions in associated nucleotides needed for DNA synthesis. The result is abnormalities in the rate and/or accuracy of mitochondrial DNA synthesis, with mitochondrial DNA depletion. Disorders associated with *TK2* are autosomal recessive, with three overlapping groups delineated by age at onset. Individuals with **infantile onset** (<1 year) have severe myopathy with proximal muscle weakness, facial diplegia, dysphagia, and respiratory compromise. Some may also have encephalopathy and seizures. Death usually occurs within 1-4 years. Individuals with the **childhood-onset** form (up to 12 years of age) have a more slowly progressive myopathy with weakness and longer survival periods. Those with **late onset** (>12 years of age) may have limited areas of weakness such as chronic progressive ophthalmoplegia, facial diplegia, or oropharyngeal weakness. There is no known treatment, but trials with relevant pyrimidine derivatives have been initiated.

COMBINED DISORDERS OF BOTH PURINES AND PYRIMIDINES

PRPS1-Associated Disorders

The *PRPS1* gene encodes the enzyme PRPP synthase type 1. Two additional isoforms of this enzyme are encoded by separate genes: *PRPS1L1* and *PRPS2*. *PRPS1* and *PRPS2* reside on opposite arms of the X chromosome, and *PRPS1L1* is on chromosome 7. *PRPS1* is ubiquitously expressed. *PRPS1L1* is expressed only in testis, and *PRPS2* is expressed in the gastrointestinal system, endocrine tissues, and reproductive organs. Only *PRPS1* has been linked with human disease. *PRPS1*-associated disorders are rare, with fewer than 1,000 individuals reported.

All three isoforms of PRPP-synthase transfer high-energy phosphate bonds from ATP to ribose-5-phosphate to generate PRPP. The PRPP can then use the high-energy phosphate bonds to drive certain energy-requiring reactions. PRPP is a cosubstrate for the first and rate-limiting reaction in purine synthesis (see Fig. 110.2). PRPP also serves as a cosubstrate for the main purine salvage enzymes HGprt and APRT. PRPP affects pyrimidine synthesis as well, because it is a cosubstrate for the first step in pyrimidine synthesis (see Fig. 110.3). PRPP is also a cosubstrate for two additional enzymes involved in the synthesis of the pyridine nucleotides: NAD and NADP (Table 110.4). PRPP-synthase plays a key role in regulating the synthesis of both purines and pyrimidines, and overexpression of *PRPS1* is common in certain cancer cells, which need large quantities of purines and pyrimidines for DNA replication during cell division.

Because of its integral involvement in many vital biochemical pathways, complete loss of PRPP-synthase is not compatible with life. Instead, clinical disease results from abnormally high enzyme activity or partial loss of enzyme activity. High levels of the enzyme produce excessive quantities of PRPP, and elevations in PRPP accelerate purine production, with resultant overproduction of uric acid. It is likely that increases in PRPP accelerate pyrimidine metabolism, but measures of this pathway are not widely used in clinical medicine. Conversely, impaired enzyme activity results in reduced metabolism of both purines and pyrimidines.

Clinical Features

The clinical phenotypes of *PRPS1*-associated disorders are remarkably diverse. Phenotypic variation is caused by varied changes in enzyme function. The phenotypes associated with *reduced enzyme activity* historically have been described as distinct entities, although clinical features overlap, and the reality is a continuous spectrum of severity (Fig. 110.5). The mildest phenotype is **X-linked nonsyndromic hearing loss (DFN2)**. Hearing impairments in males may range from a slowly progressive postlingual hearing loss to profound congenital deafness. A more severe phenotype associated with impaired enzyme activity has been called **Rosenberg-Chutorian syndrome or X-linked Charcot-Marie-Tooth disease (CMTX5)**. This phenotype in males combines prelingual hearing loss, progressive optic neuropathy, and peripheral neuropathy beginning at 5-10 years of age and sometimes gait impairments. An even more severe phenotype has been called **Arts syndrome**. It also is X-linked, and males have profound congenital deafness, early-onset optic neuropathy, peripheral neuropathy, psychomotor delay, and recurrent respiratory infections that may cause early death. In addition to these three phenotypes classically associated with reduced enzyme activity, there are reports that suggest a fourth and even more severe phenotype. Males have intrauterine growth restriction and failure to thrive after birth, congenital retinopathy and deafness, diabetes insipidus, and a more severe neurologic condition that includes severe psychomotor delay, spastic tetraparesis, seizures, and evidence for delayed white matter development on brain MRI.

The *PRPS1* gene is X-linked, so variants associated with reduced PRPP-synthase affect males, often from an early age. However, female carriers may express milder or adult-onset phenotypes. In families where males are affected only with early-onset hearing loss, female carriers may develop progressive hearing loss as young adults. In

families where males have a more severe early childhood phenotype that includes additional neurologic signs, female carriers may have progressive hearing loss, retinopathy, optic neuropathy, peripheral neuropathy, and sometimes additional signs attributable to the central nervous system. For the most severe phenotype, female carriers may also have short stature. In some families, only females are clinically affected, with evidence for embryonic lethality of males. These families suggest the existence of a fifth phenotype of embryonic lethality in males. Because hearing loss is the most consistent feature associated with reduced PRPP-synthase, the diagnosis of a *PRPS1*-associated disorder should be considered in males or females with sensorineural hearing loss, especially when combined with other neurologic signs.

There are also two different phenotypes associated with *abnormally high levels* of PRPP-synthase activity. The milder phenotype is associated with overexpression of a normal *PRPS1* mRNA transcript. Males present with signs of uric acid overproduction, including hyperuricemia and gout. Female carriers may also be affected. A more severe phenotype is associated with a point pathogenic variant in *PRPS1*, which renders the protein less durable but insensitive to feedback inhibition. The lack of feedback inhibition results in excessive PRPP production, with acceleration of purine synthesis and overproduction of uric acid. However, tissues with normally low levels of *PRPS1* may experience PRPP shortage because the enzyme is unstable. Erythrocytes lack a nucleus, so there is no ongoing mRNA transcription to provide a constant supply of new enzyme. In individuals with an unstable enzyme, erythrocyte PRPP-synthase is absent. A similar mechanism may explain the neurologic consequences, which overlap with those of PRPP-synthase deficiency syndromes. Affected individuals show signs of overproduction of uric acid combined with sensorineural hearing impairments, neuropathy, and/or psychomotor delay. The diagnosis of a disorder associated with excessive activity of PRPP-synthase should be suspected in any individual with evidence for overproduction of uric acid, especially when combined with the typical neurologic signs.

Treatment and Prognosis

The overproduction of uric acid in disorders associated with increased activity of PRPP-synthase is treated with inhibitors of XOR (allopurinol or febuxostat) combined with generous hydration. No treatments have proven effective in the treatment of disorders associated with reduced activity of the enzyme.

Disorders Associated with Nucleotidases

There are numerous enzymes that function as nucleotidases to remove phosphate groups from nucleotides (see Figs. 110.1 and 110.3). Many act nonselectively on multiple nucleotides and other small molecules, but they are often described according to their most prominent (or first discovered) enzymatic activity. At least five of these nucleotidases are cytosolic, one is bound to the plasma membrane, and another is localized to mitochondria. Early studies based on enzymatic activity did not always identify the exact isoform involved, but more recent studies have more precisely delineated the precise enzyme involved.

Nucleotidase-associated pervasive developmental delay. A marked increase in nucleotidase activity was found in cells of nine individuals with motor and cognitive delay, hyperactivity and impulsivity, delayed or absent language, seizures, awkward movements, and other abnormal behaviors. Some had immunologic impairments too. Although the exact enzyme and gene were not delineated, individuals had chronic hypouricemia, indicating reduced purine metabolism. Biochemical studies suggested the disorder was associated with depletion of pyrimidine nucleotides, and the behavioral abnormalities responded to uridine supplements.

Pyrimidine 5′-nucleotidase deficiency. The *NT5C3A* gene encodes an erythrocyte-specific 5′-nucleotidase isoform (P5N-1) with preferential activity toward the pyrimidines CMP and UMP (see Fig. 110. 3). Enzyme deficiency is inherited in an autosomal recessive manner and associated with accumulation of pyrimidine nucleotides in erythrocytes.

The clinical manifestations include chronic nonspherocytic hemolytic anemia; marked basophilic stippling and reticulocytosis; and accompanying splenomegaly, hemoglobinuria, and jaundice resulting from overproduction of bilirubin. The diagnosis is suspected when there is prominent basophilic stippling of erythrocytes, a phenomenon also associated with lead intoxication. Erythrocytes have an overabundance of pyrimidines, and the enzyme can be measured in red cells. The anemia is generally moderate and does not usually require transfusion.

Spastic paraplegia (SPG45). The *NT5C2* gene encodes a cytosolic 5′-nucleotidase with preferential activity toward IMP and other purine nucleotides. Pathogenic variants in this gene leading to reduced enzyme function have been linked with spastic paraplegia (SPG45). The disorder is rare and described for fewer than 20 individuals in a few different families. Affected individuals have early-onset gait impairment with leg weakness, spasticity, hyperreflexia, clonus, and extensor plantar reflexes. Many also have cognitive impairments. MRI may show white matter brain changes with dysgenesis of the corpus callosum.

Desbuqois dysplasia type 1 (DBQB1). The *CANT1* gene encodes a secreted calcium-dependent enzyme that functions as a triphosphatase or diphosphatase with activity toward ATP, ADP, UTP, and UDP. Pathogenic variants in this gene have been linked with a rare form of osteochondrodysplasia. Affected individuals have short stature with short limbs, joint laxity with frequent joint dislocations, pectus carinatum, osteopenia, dysmorphic facies, and intellectual disability.

Table 110.4 Enzymes Dependent on Phosphoribosylpyrophosphate

ENZYME	GENE	PATHWAY
Amido phosphoribosyl transferase	*PPAT*	Purine synthesis
Hypoxanthine-guanine phosphoribosyl transferase	*HPRT1*	Purine recycling
Adenine phosphoribosyl transferase	*APRT*	Purine recycling
Orotate phosphoribosyl transferase Uridine monophosphate synthetase	*UMPS*	Pyrimidine synthesis
Nicotinate phosphoribosyl transferase	*NAPRT*	NAD synthesis
Nicotinamide phosphoribosyl transferase	*NAMPT*	NADP synthesis

NAD, Nicotinamide adenine dinucleotide; NADP, nicotinamide-adenine dinucleotide phosphate.

DISORDERS ASSOCIATED WITH RIBONUCLEOTIDE REDUCTASE

Ribonucleotide reductase is the enzyme responsible for generating the deoxynucleotides dADP, dCDP, dGDP, and dUDP (see Figs. 110.1 and 110.3). These deoxynucleotides are used to synthesize DNA, so the enzyme plays a key role in regulating DNA replication for cell division. The enzyme is the target for hydroxyurea used for sickle cell anemia and certain malignancies. The human enzyme has three subunits, but only the subunit encoded by the *RRM2B* gene has been linked with human disease.

Clinical Features

Pathogenic variants in *RRM2B* are responsible for a **mitochondrial DNA depletion syndrome** with severe encephalomyopathy. The disorder is inherited in an autosomal recessive manner. Only a handful of cases from a few families have been reported. Pathogenic variants in the same gene have also been linked with an autosomal dominant **progressive external ophthalmoplegia (PEOA5)**. Only a few cases have been reported. There is no specific treatment for either disorder.

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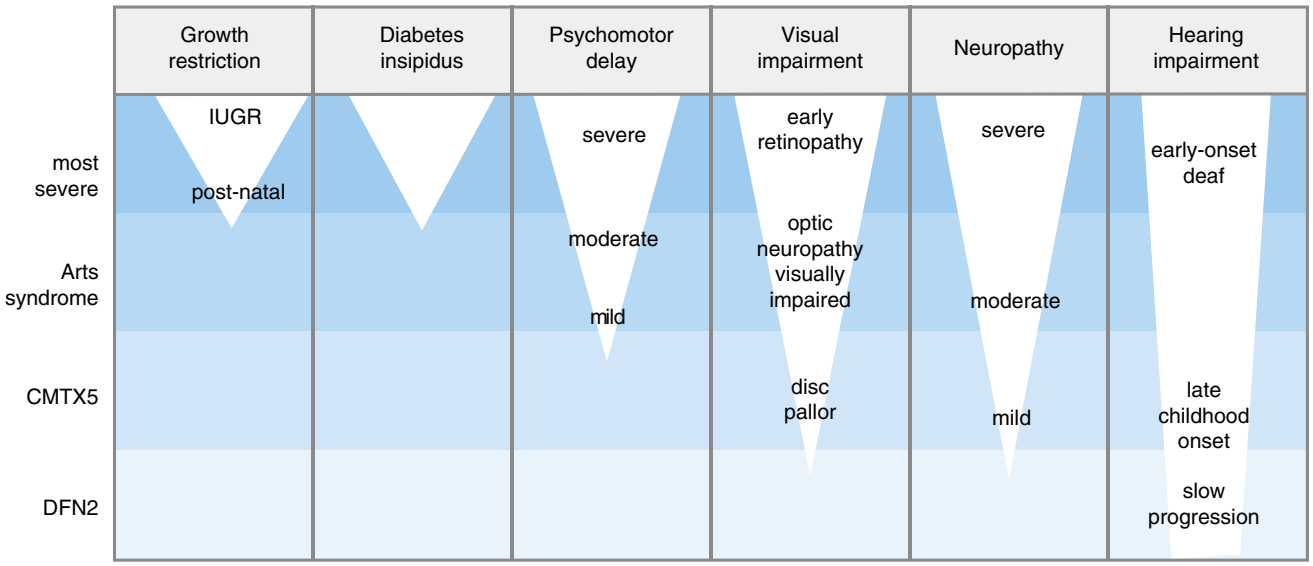

Fig. 110.5 *PRPS1*-associated disorders. This schematic provides a graphical representation of the overlapping spectrum of *PRPS1*-associated disorders. The four disorders are listed on the left side of the panel. The most common clinical features are shown in the top row. The *white triangles* depict the severity of individual clinical features for each of the disorders. For example, hearing loss may begin in adulthood with slow progression in the milder phenotypes (DFN2), whereas it may be reflected as earlier or more complete deafness in the more severe phenotypes. Conversely, growth retardation and diabetes insipidus have been reported only for the most severe phenotype. This schematic provides only a general guide, as some exceptions may occur. (*Copyright H.A. Jinnah.*)

Chapter 111

Hutchinson-Gilford Progeria Syndrome (Progeria)

Timothy R. O'Toole and Leslie B. Gordon

Hutchinson-Gilford progeria syndrome (HGPS, or progeria) is a rare, fatal, autosomal dominant segmental premature aging disease. With an estimated incidence of 1 in 4 million live births and prevalence of 1 in 20 million living individuals, there are an estimated total of 400 children living with progeria in 2023 worldwide. There is no gender, ethnic, or regional bias.

Progeria is caused by a single-base pathogenic variant in the *LMNA* gene, which results in the production of an abnormal lamin A protein called **progerin**. Lamin A is an intermediate-filament inner nuclear membrane protein found in most differentiated cells of the body. Without progerin-directed treatment, children with progeria develop **premature progressive atherosclerosis** and die of heart failure, usually between ages 5 and 20 years. Progerin is found in increased concentration in the skin and vascular wall of normal older individuals compared with younger individuals, suggesting a role in normal aging.

CLINICAL MANIFESTATIONS

Children are born looking normal and begin to develop clinical signs of disease during year one. Physical appearance and clinical findings change dramatically each year that they age (Figs. 111.1 and 111.2). Both clinical and biologic overlaps with aging are segmental, or partial. The disease features discussed next are roughly in order of clinical appearance.

Dermatologic Changes in Skin, Hair, and Nails

Skin findings are often apparent as initial signs of progeria—at birth in about 25% of cases and by age 2 months in 80% of cases. These are variable in severity and include areas of discoloration, stippled dyspigmentation, tightened areas that can restrict movement, and areas of the trunk or legs where small (1-2 cm), soft, bulging skin is present.

Although usually born with normal appearance, cranial hair is lost within the first few years. Initial hair loss occurs in the temporal and occipital areas, with preservation of hair on the mid-scalp and vertex for the longest period. Eventually total alopecia occurs, leaving soft, downy, sparse, immature hair on the scalp, no eyebrows, and scant eyelashes.

Nails on hands and feet are usually normal at birth but become dystrophic later in life.

Failure to Thrive

Children with progeria experience apparently normal fetal and early postnatal development. Between several months and 1 year of age, abnormalities in growth and body composition are readily apparent. Severe failure to thrive ensues, heralding generalized lipoatrophy, with apparent wasting of limbs, circumoral cyanosis, and prominent veins around the scalp, neck, and trunk resulting from a paucity of subcutaneous fat. The weight percentile is usually normal at birth but decreases to below the third percentile despite adequate caloric intake for normal growth and normal resting energy expenditure. A review of 35 children showed an average weight increase

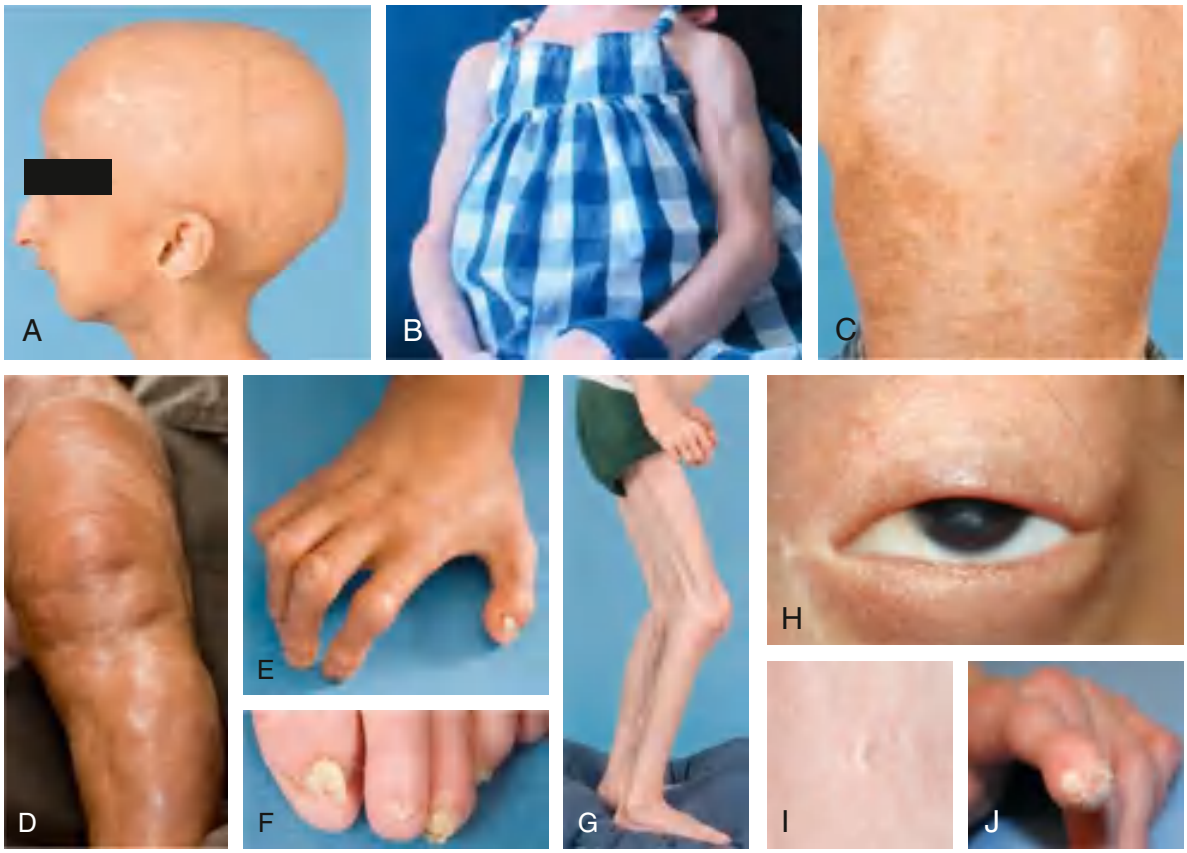

Fig. 111.1 Distinguishing clinical features in Hutchinson-Gilford progeria syndrome. **A,** Alopecia, prominent scalp veins, narrowed nasal bridge, retrognathia. **B,** Generalized lipoatrophy leaves muscular prominence. **C,** Skin tightening and mottling. **D,** Skin bulging. **E,** Digital joint contractures. **F,** Nail dystrophy with spooning. **G,** Knee joint contractures, lipodystrophy. **H,** Corneal scarring secondary to exposure keratopathy. **I,** Flat umbilicus with scarred-over appearance; **J,** Calcinosis cutis in a digit. (*Photos courtesy The Progeria Research Foundation and Boston Children's Hospital.*)

of only 0.44 kg/year, beginning at 24 months of age and persisting through life. Weight gain over time is linear, which contrasts with the pulsatile acceleration in growth velocity for normal age- and gender-matched children. Children reach an average final height of approximately 1 meter and weight of approximately 15-20 kg. Head circumference is normal. The weight deficit is more pronounced than the height deficit and, associated with the loss of subcutaneous fat, results in the emaciated appearance with muscular prominence. Clinical problems caused by the lack of subcutaneous fat include sensitivity to cold temperatures and foot discomfort caused by lack of fat cushioning. Overt diabetes is unusual in progeria, but at least 75% of children eventually develop insulin resistance, usually starting at around age 8 years.

Musculoskeletal Impairments

Both upper and lower extremity range-of-motion impairments (e.g., fingers, elbows, hips, knees, ankles) may be present at birth and may progress with age. Joint contractures are caused by both bony and cartilaginous disease, along with tightened skin. Along with irregularities in the congruency of articulating joint surfaces, these changes serve to limit joint motion and affect both upper and lower extremity gross and fine motor function. *Physical therapy is recommended routinely and throughout life to maximize joint function.*

Ocular Abnormalities

Ophthalmic signs and symptoms are caused in part by shallow orbits, tight skin, and a paucity of subcutaneous fat around the eyes. Eyes are prominent and often experience ocular surface disease secondary to nocturnal lagophthalmos and exposure keratopathy, which produce photophobia, tearing, ocular irritation, corneal scarring, and corneal ulceration that can lead to vison loss. Most patients have relatively good acuity; however, advanced ophthalmic disease can be associated with reduced acuity. *Children with progeria should have an ophthalmic evaluation at diagnosis and at least yearly thereafter.* Aggressive ocular surface lubrication is recommended, including the use of tape tarsorrhaphy at night.

Craniofacial and Dental Phenotypes

Children develop craniofacial disproportion, with micrognathia and retrognathia caused by mandibular hypoplasia. Typical oral and dental manifestations include hypodontia, delayed tooth eruption, severe dental crowding caused by hypoplastic maxilla and mandible, ogival palatal arch, ankyloglossia, presence of median sagittal palatal fissure, and generalized gingival recession. Eruption may be delayed for many months, and primary teeth may persist for the duration of life. Secondary teeth are present but may or may not erupt. They sometimes erupt on the lingual and palatal surfaces of the mandibular and maxillary alveolar ridges, rather than in place of the primary incisors. In some, but not all cases, extracting primary teeth promotes movement of secondary teeth into place.

Skeletal Abnormalities

Development of bone structure and bone density represents a unique skeletal dysplasia that is not based in malnutrition. Acroosteolysis of the distal phalanges, distal clavicular resorption, and thin, tapered ribs are early signs of progeria (as early as 3 months

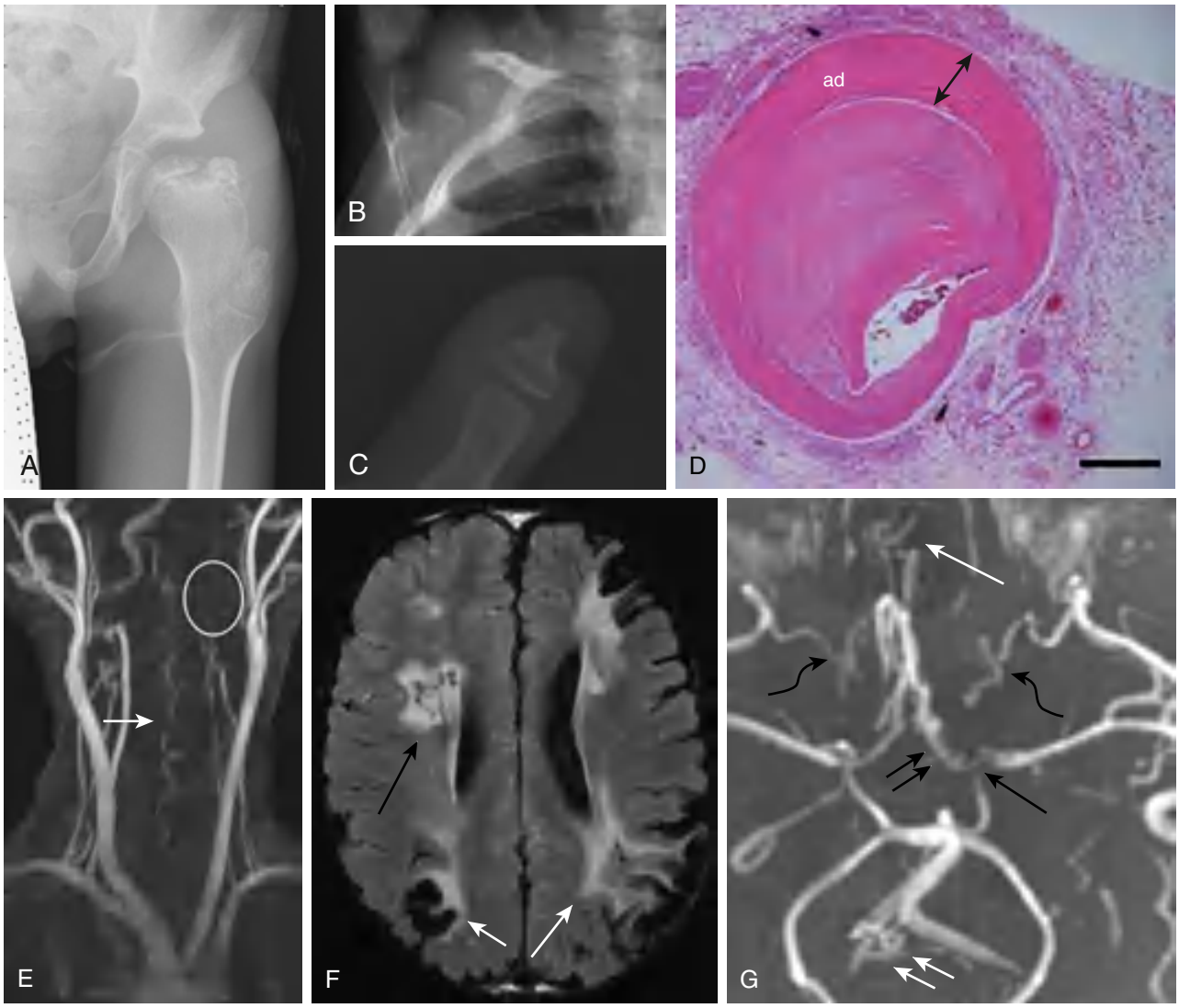

Fig. 111.2 Pathologic skeletal and cardiovascular findings in Hutchinson-Gilford progeria. **A,** Coxa valga of the hip. **B,** Clavicular osteolysis. **C,** Acroosteolysis in a thumb. **D,** H&E staining of mid-right coronary artery characterized by an enlarged and highly fibrotic adventitia *(arrow).* The media is markedly thinned in the area with adventitial fibrosis and plaque showing clinically significant stenosis (90%) and a necrotic core with calcification (staining not shown). **E,** Near-complete loss of the distal vertebral artery *(circle)* and prominent anterior spinal artery collateral *(arrow).* **F,** Acute gyral infarcts *(black arrow).* Bright signal in the sulci indicates slow cortical collateral flow *(white arrows).* **G,** M1 *(single black arrow)* and A1 stenoses *(double black arrows),* internal maxillary artery collaterals *(wavy black arrows),* subfrontal collaterals *(single white arrow),* and enlarged anterior and posterior spinal arteries *(double white arrows).*

of age). *Facial disproportion, a narrowed nasal bridge, and retrognathia make intubation extremely difficult, and fiberoptic intubation is recommended.* A pyriform chest structure and small clavicles can lead to reducible glenohumeral joint instability. Growth of the spine and bony pelvis is normal. Hip disease is pervasive and results in repeated dislocations in about 20% of cases that are not amenable to splinting. Dysplastic growth of the femoral head and neck axis results in coxa valgus (straightening of the femoral head-neck axis >125 degrees) and coxa magna, where the diameter of the femoral head is disproportionately large for the acetabulum, resulting in hip instability. The resulting hip dysplasia can be progressive and may result in osteoarthritis, avascular necrosis, hip dislocation, and inability to bear weight.

In response to repeated hip dislocations, five instances of either unilateral or bilateral surgical correction of hip dysplasia using periacetabular and femoral osteotomy have been conducted, each at different hospitals. Follow-up after 4-8 years has yielded mixed results. Other than in a single instance, hip dislocations have not recurred. In two cases, postsurgical healing occurred normally, resulting in ability to bear weight and walk short-to-long distances. In two cases, hardware failure with nonunion of femoral bone necessitated hardware removal and new hardware insertion; skin breakdown and need for additional bone grafting complicated one of these cases. These two cases yielded limited weight-bearing with no assistance when walking short distances, but the patients required wheelchair use for longer distances. Finally, one case healed well with no adverse events, but mobility was not restored because of postsurgery progression of joint contractures, resulting in inability to bear weight or walk and subsequent wheelchair use. Adequate vitamin D levels are likely important for bone healing in all cases.

Other changes to the appendicular skeleton include flaring of the humeral and femoral metaphyses and constriction of the radial neck. Growth plate morphology is generally normal but can be variable within a single radiograph. The appearance of ossification

centers used to define bone age is normal. Bone structure assessed by peripheral quantitative computed tomography (pQCT) of the radius demonstrates distinct and severe abnormalities in bone structural geometry, consistent with progeria representing a *skeletal dysplasia*. Areal bone mineral density (aBMD) *z* scores measured by dual-energy x-ray absorptiometry (DXA) adjusted for height and age and true (volumetric) BMD assessed by pQCT are normal to mildly reduced, refuting the assumption that patients with progeria are osteoporotic. Fracture rates in progeria are normal and not associated with fragility fractures observed in other pediatric metabolic bone diseases, such as osteogenesis imperfecta.

Hearing

Low-tone conductive hearing loss is pervasive in progeria and likely indicative of a stiff tympanic membrane and/or deficits in the middle ear bony and ligamentous structures. Overall, this does not affect the ability to hear the usual spoken tones, but preferential classroom seating is recommended, along with annual hearing examinations.

Cardiovascular Disease

Approximately 80% of deaths in progeria are caused by cardiovascular failure, with end-stage events sometimes precipitated by superimposed respiratory infection or stressors surrounding surgical intervention. Progeria is a **primary vasculopathy** characterized by pervasive accelerated vascular stiffening, followed by large- and medium-vessel occlusive disease from atherosclerotic plaque formation, with valvular and cardiac calcification and insufficiency at advanced stages of disease. Some evidence of vascular dysfunction is apparent in all ages tested. ECG abnormalities are generally nonspecific, but indications of left ventricular hypertrophy and nonspecific ST-T wave abnormalities can occur later in disease progression. Hypertension, angina, cardiomegaly, metabolic syndrome, and congestive heart failure are common end-stage events.

Transthoracic echocardiography reveals early-onset diastolic left ventricular dysfunction associated with age-related decline in lateral and septal early (E′) diastolic tissue Doppler velocity *z* scores and an increase in the ratio of mitral inflow (E) to lateral and septal E′ velocity *z* scores. Other echocardiographic findings include left ventricular hypertrophy, left ventricular systolic dysfunction, and mitral or aortic valve disease with calcification, which tend to appear later in life.

Vascular stiffening assessed using carotid and femoral ultrasound reveals elevated carotid-femoral pulse wave velocity (PWV_{cf}) and wall echodensity with medial and adventitial thickening. These are considered early and important indicators of cardiovascular decline. In the general population, increased PWV_{cf} is associated with increased risk of cardiac death. Intima-media thickness is normal. In addition, elevated carotid artery mean flow velocities appear early in life, and underscore the presence of arterial occlusive disease.

Routine blood pressure (BP) monitoring with special attention to proper cuff size is recommended. When normalized for patient size using height and age correction, both systolic and diastolic BP are increased in about half of patients with HGPS. These elevated BP trends would be expected in the setting of significant vascular stiffness. *Antihypertensive medications have been used in the setting of elevated BP, with special attention to maintaining adequate BP to avoid strokes.*

BP monitoring, ECG, echocardiography, carotid ultrasound for plaque evaluation, and PWV_{cf} measures for vascular stiffening are recommended.

End-stage cardiovascular disease (CVD) in progeria is often characterized by atherosclerotic plaques in large and medium-sized arteries, severe aortic stenosis, and cardiac failure. Critical aortic stenosis with heart failure is amenable to transapical transcatheter aortic valve implantation and apico-aortic conduit surgeries to alleviate severe aortic stenosis. These carry high risk of death but have yielded significant symptomatic improvement and prolongation of life in several cases (see treatment section).

Cerebrovascular Arteriopathy and Stroke

The earliest incidence of stroke occurred at age 0.4 years. More often, strokes occur in the later years. Over the life span, MRI evidence of infarction can be found in as many as 60% of patients with progeria, with half of these clinically silent. Both extracranial and intracranial occlusive disease is present in the neurovascular axis, with resulting extensive collateral vessel formation that pseudonormalizes intracranial flow. Carotid artery blockages are well documented, but infarction can occur even in their absence. Both large- and small-vessel disease is found; collateral vessel formation is extensive. A propensity for strokes and an underlying stiff vasculature make maintaining adequate blood pressure through oral hydration a priority in patients with progeria; special care should be taken when considering maintenance of consistent BP during general anesthesia, airplane trips, and hot weather. In addition, 15% of deaths in children with progeria occur from head injury or trauma, including subdural hematoma. This implies an underlying susceptibility to subdural hematoma, though known risk of stroke is much higher and frequently warrants low-dose aspirin therapy.

Sexual Development

Females with progeria can develop Tanner stage II secondary sexual characteristics, including signs of early breast development and sparse pubic hair. Progression to Tanner stage III has not been observed. Despite minimal to absent physical signs of pubertal development and markedly reduced body fat, over half of females experience spontaneous menarche at a median age of 14 years. Menarche is not preceded by gain in body mass or increase in body fat. Timing of menarche is variable; some adolescents start menstruation at Tanner stage I without progression to Tanner II, while others start at Tanner stage II. Those experiencing menarche vs nonmenstruating females have similar body mass indices, percentage body fat, and serum leptin levels, all of which are vastly below the healthy adolescent population. Menorrhagia has been observed and can result in symptomatic anemia. *In addition to iron supplementation, oral contraceptives may be indicated to regulate menses.* Progestin-only or minimal estrogen-content products are preferred because of elevated cardiovascular risks associated with estrogen-based medication in the face of HGPS-associated atherosclerosis. There are no documented cases of reproductive capacity in females or males with progeria; however, the data suggest there is potential for further sexual development and the possibility of fertility in these females. Secondary sexual characteristics in males have not been reported.

Normally Functioning Systems

Liver, kidney, thyroid, immune, gastrointestinal, and neurologic function (other than stroke related) remain intact. Intellect is normal for age, possibly in part from downregulation of progerin expression in the brain by a brain-specific micro-RNA, miR-9.

LABORATORY FINDINGS

The most consistent laboratory findings are low serum leptin below detectable levels (>90%) and insulin resistance (75%, usually starting over age 8 years). Platelet count is often moderately high. High-density lipoprotein (HDL) cholesterol and adiponectin concentrations decrease with increasing age to values significantly below normal. Otherwise, lipid panels, high-sensitivity C-reactive protein, blood chemistries, liver and kidney function tests, endocrine test, and coagulation tests are generally normal.

MOLECULAR PATHOGENESIS

Pathogenic variants in the *LMNA* gene cause progeria. The normal *LMNA/C* gene encodes the proteins lamins A and C, of which only lamin

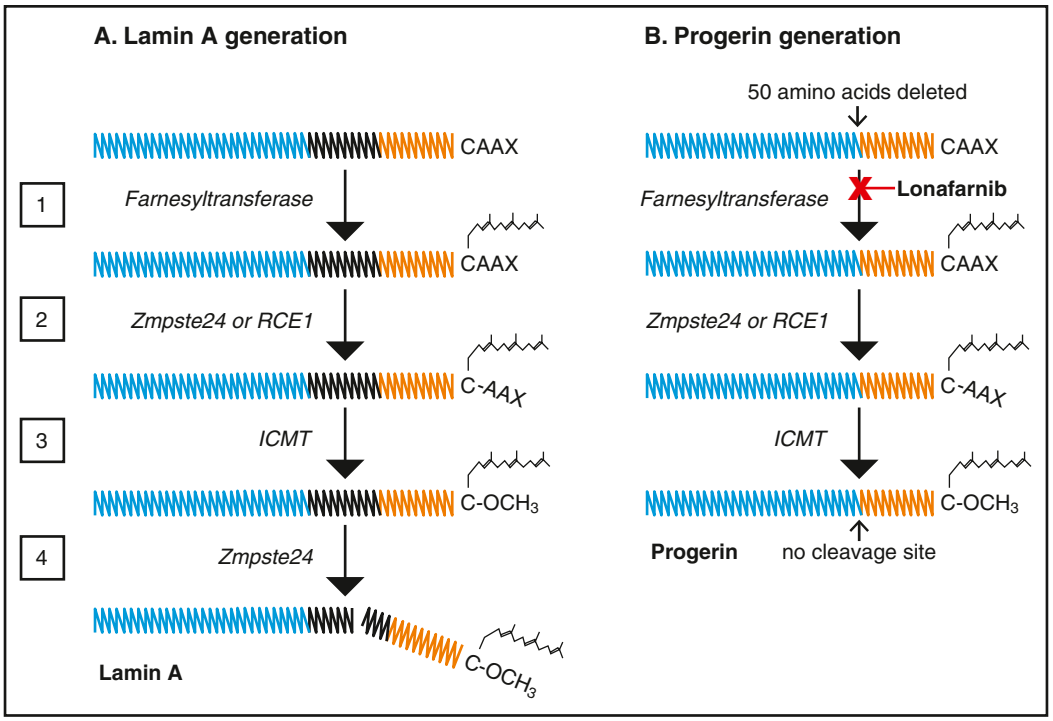

Fig. 111.3 Posttranslational processing pathways producing lamin A and progerin, including the target site for lonafarnib. **A,** Prelamin A polypeptide chain, showing its central α-helical rod domain and C-terminal–CAAX box, representing cysteine (C), aliphatic amino acids (AA), and any amino acid (X). The α-helical rod domain is divided into segments that assist in displaying the progerin defect. Posttranslational processing consists of four steps: (1) a farnesyl group is attached to the cysteine residue of the −CAAX box by farnesyltransferase; (2) the last three residues are proteolytically cleaved by the zinc metalloprotease Zmpste24 or Ras-converting enzyme (RCE1); (3) carboxy-methylation by isoprenyl-cysteine carboxyl methyltransferase (ICMT); and (4) the terminal 15 C-terminal residues, including the farnesylated and carboxymethylated cysteine, are cleaved off by Zmpste24. **B,** A 50–amino acid deletion in prelamin A (represented by the black segment of the lamin A rod) is the result of a pathogenic variant that activates a cryptic splice site within exon 11 of the *LMNA* gene. This deletion leaves progerin without an attachment site for the last processing step—cleavage of the farnesylation and carboxymethylated terminal 15 amino acid residues. Thus progerin remains farnesylated and intercalated within the inner nuclear membrane, where it causes much of its cellular damage.

A is associated with human diseases. The lamin proteins are the principal proteins of the nuclear lamina, a complex molecular interface located between the inner membrane of the nuclear envelope and chromatin. The integrity of the lamina is central to many cellular functions, creating and maintaining structural integrity of the nuclear scaffold, DNA replication, RNA transcription, organization of the nucleus, nuclear pore assembly, chromatin function, cell cycling, senescence, and apoptosis.

Progeria is a sporadic autosomal dominant disease in about 98% of cases. Two percent of cases presumably arise from parental mosaicism; The Progeria Research Foundation reports five identified sibling occurrences of genetically confirmed HGPS. HGPS is caused by the accelerated use of an alternative, internal splice site that results in the deletion of 150 base pairs in the 3′ portion of exon 11 of the *LMNA* gene. In about 90% of cases, this results from a single C to T transition at nucleotide 1824 that is silent (Gly608Gly) but optimizes an internal splice site within exon 11. The remaining 10% of cases possess one of several single-base pathogenic variants within the intron 11 splice donor site, thus reducing specificity for this site and altering the splicing balance in favor of the internal splice. Subsequent to all these pathogenic variants, translation followed by posttranslational processing of the altered mRNA produces progerin, a shortened abnormal lamin A protein with a 50–amino acid deletion near its C-terminal end. An understanding of the posttranslational processing pathway and how it is altered to create progerin has led to a number of treatment prospects for the disease (Fig. 111.3).

Both prelamin A and preprogerin possess a methylated farnesyl side group attached during posttranslational processing. This is a lipophilic moiety that facilitates intercalation of proteins into the inner nuclear membrane, where most of the lamin and progerin functions are performed. During posttranslational processing of normal lamin A, loss of the methylated farnesyl anchor releases prelamin from the nuclear membrane, rendering it soluble for autophagic degradation. However, preprogerin and, subsequently, progerin retain the farnesyl moiety. Progerin remains anchored to the membrane, binding other proteins, causing blebbing of the nucleus, disrupting mitosis, and altering gene expression. Progerin also retains a methyl moiety.

Disease in progeria is produced by a dominant negative mechanism; *the action of progerin,* not the diminution of lamin A, causes the disease phenotype. The severity of disease is determined in part by progerin levels, which are regulated by the particular pathogenic variant, tissue type, or other factors influencing use of the internal splice site.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Overall, the constellation of small body habitus, bone, hair, subcutaneous fat, and skin changes results in the marked physical resemblance among patients with progeria (Fig. 111.4). For this reason, if disease signs are present, clinical diagnosis can often be achieved or excluded with relative confidence even at young ages. There are rare

Fig. 111.4 Unrelated 7-yr-old female and 10-yr-old male with progeria. The appearance is remarkably similar between patients. (*Photograph courtesy The Progeria Research Foundation.*)

cases of mosaicism or relatively low-progerin-expressing patients with extremely mild clinical signs of disease. Thus lack of early disease manifestation should not be considered exclusionary. Clinical suspicion should be followed by *LMNA* genetic sequence testing. In addition, a number of premature aging disorders have features that overlap with HGPS but are not progerin-producing (Table 111.1). Patients may fall under none of these diagnoses and represent ultrarare, unnamed progeroid diseases that carry either non–progerin-producing pathogenic variants in *LMNA* or the lamin-associated enzyme (ZMPSTE24), or progeroid syndromes without lamin-associated pathogenic variants.

TREATMENT

Lonafarnib (Zokinvy) is a farnesyltransferase inhibitor that blocks the addition of a farnesyl lipid moiety onto preprogerin, thus preventing progerin from associating with the inner nuclear membrane where it effects much of its damage (see Fig. 111.3). Lonafarnib is approved by the US FDA and European Medicines Agency for use in patients with HGPS and processing-deficient progeroid laminopathies ages 12 months and older. It is the first and only approved indication for either disease. It is initiated at a dose of 115 mg/m^2 orally twice daily. After four months, the dose is increased to 150 mg/m^2 twice daily. The most common side effects are diarrhea, nausea, and loss of appetite, which can be mitigated with loperamide, ondansetron HCl, and cyproheptadine, respectively.

Overall, children with HGPS treated with long-term lonafarnib therapy demonstrated an increase in mean estimated survival of 4.3 years (30%) over untreated children. Treatment decreased plasma progerin levels by an average of 38% starting at 4-6 months and persisting for up to 10 years on therapy. In a single-arm clinical trial with lonafarnib (NCT00425607), subgroups of patients experienced increased rate of weight gain, decreased vascular stiffness measured by decreased PWV_{cf} and carotid artery echodensity, improved left ventricular diastolic function, increased radial bone structural rigidity, improved sensorineural hearing, and early evidence of decreased headache, TIA, and stroke rates. Dermatologic, dental, joint contracture, insulin resistance, lipodystrophy, BMD, and joint contractures were unaffected by drug treatment.

Dosing: In general, medications should be dosed according to weight or body surface area instead of age because of the small size and decreased weight for height.

Hydration is important for maintaining adequate blood flow in the face of generalized vascular stiffness and collateral vascular formation in the brain.

Low-dose aspirin therapy is recommended at 2 mg/kg/day, as an extension of what is known about decreasing cardiovascular risk in the general at-risk adult population. It is not known whether low-dose aspirin therapy has any effect on morbidity or mortality in HGPS.

Antihypertensive medications have been used with elevated BP, with special attention to maintaining adequate BP to avoid strokes. Due to small, thin body habitus, appropriately sized blood pressure cuff and height-age adjustments when evaluating hypertension (>95th percentile) should be employed.

Extraskeletal calcifications have been observed in patients with progeria radiographically and cutaneously, both at a baseline off therapy (around 30% of patients) and with increasing frequency during a clinical trial when the patients were treated with oral calcium carbonate supplementation, and/or zoledronic acid, pravastatin, and lonafarnib (around 45% of patients). Oral calcium carbonate supplementation may therefore aggravate calcium dysfunction in children with progeria. Given this potential concern, calcium intake via dietary means, along with vitamin D supplementation, is likely the safest intake strategy.

Physical and occupational therapy initiation are recommended as young as possible to preserve joint mobility and optimize capacity for activities of daily living.

Cardiac intervention. A primary cause of mortality in HGPS is critical aortic stenosis caused by premature atherosclerosis. At end stage, high-risk intervention has been performed using transcatheter aortic valve implantation (TAVI) for patients large enough to receive the smallest valve available, using a transapical approach because the femoral artery is small and calcified, precluding a transfemoral approach. The majority of patients' aortic valves are too small to implement TAVI. In these cases, apico-aortic conduit (aortic valve bypass) surgery has been achieved. Both procedures have resulted in postoperative symptomatic relief and lifespan extension when successful. The procedures carry high risk for perisurgical morbidity and mortality.

Clinical trials. One currently ongoing clinical trial adding *everolimus* (an FDA-approved mTOR inhibitor) to a lonafarnib regimen is aimed at accelerating autophagy of progerin, thus theoretically reducing its accumulation and cellular damage (NCT02579044). Patient treatment phase is complete and results are pending.

PROGNOSIS

Children with progeria develop a severe premature form of atherosclerosis. Before death, cardiac decline with left-sided hypertrophy, valvular insufficiency, and pulmonary edema develop; neurovascular decline with TIAs, strokes, and occasionally seizures can result in significant morbidity.

Without lonafarnib drug treatment, death occurs generally between ages 5 and 20 years, with a median life span of 14.5 years, resulting from heart failure, sometimes with superimposed respiratory infection (approximately 80%); from head injury or trauma, including subdural hematoma (approximately 15%); and, rarely, from stroke (1–3%) or complications from anesthesia during surgery (1–3%). With lonafarnib therapy, long-term use has been associated with up to 4.3 years' (30%) lifespan extension. Surgical intervention for critical aortic stenosis can decrease cardiovascular morbidity and extend lifespan by an indeterminate duration.

PATIENT RESOURCES

The Progeria Research Foundation (www.progeriaresearch.org) maintains an international progeria patient registry, provides a diagnostics program and complete patient care manual, and coordinates clinical treatment trials. It funds preclinical and clinical research to define the underpinnings of the disorder and to discover

Table 111.1 Features of Hutchinson-Gilford Progeria Syndrome and Other Premature Aging Disorders with Overlapping Features

DISEASE	CAUSATIVE GENE	ONSET	MAIN CLINICAL FEATURES
Hutchinson-Gilford progeria syndrome (HGPS)	*LMNA*: de novo dominant point pathogenic variant (c.1824C>T; p.G608G or pathogenic variants in first five intronic bases of intron 11)	Early childhood	Severe failure to thrive in infancy, progressive alopecia leading to total alopecia, skin lesions, characteristic facies, loss of subcutaneous fat, bone changes, skeletal anomalies, musculoskeletal degeneration, hearing loss, high-pitched voice, delayed and crowded dentition, atherosclerosis, cerebrovascular disease, average death in mid-teens from myocardial infarction or stroke (from cardiovascular disease).
Restrictive dermopathy (RD)	*ZMPSTE24*: recessive null pathogenic variants	Neonatal	Intrauterine growth restriction, reduced fetal movements, and preterm delivery, tight and translucent skin with erosions, facial dysmorphism, skeletal malformations, generalized arthrogryposis; lethal within the first weeks of life
Mandibuloacral dysplasia type B (MADB)	*ZMPSTE24*: recessive pathogenic variants: often compound heterozygous pathogenic variants with a null allele and one maintaining some residual activity	Early childhood	Generalized lipodystrophy, altered skin pigmentation, alopecia, severe bone and growth defects
Mandibuloacral dysplasia type A (MADA)	*LMNA*: recessive missense pathogenic variants	Early childhood	Partial lipodystrophy at torso and limbs, bone abnormalities, altered skin pigmentation, lipodystrophic signs and mildly accelerated aging
Nestor-Guillermo progeria syndrome (NGPS)	*BANF1*: recessive pathogenic variant (c.34G>A; p.Ala12Thr)	Early childhood	Failure to thrive, aged appearance, growth restriction, decreased subcutaneous fat, thin limbs, stiff joints, severe osteolysis, absence of early cardiovascular impairment
Mandibular hypoplasia, deafness, progeroid features, and lipodystrophy syndrome (MDPL)	*POLD1*: dominant pathogenic variants, including the common de novo pathogenic variant (c.1812_1814delCTC. P.Ser605del) observed in 80% of the patients	Early childhood	Mandibular hypoplasia, prominent loss of subcutaneous fat, progeroid appearance, skin abnormalities, metabolic abnormalities including insulin resistance and diabetes mellitus, sensorineural deafness, hypogonadism in males
Mandibuloacral dysplasia associated with *MTX2* (MADaM)	*MTX2*: recessive pathogenic variants	Early childhood	Small viscerocranium with mandibular underdevelopment, growth restriction, lipodystrophy, altered skin pigmentation, distal acroosteolyses, renal focal glomerulosclerosis, severe cardiovascular disease
Atypical progeroid laminopathies	*LMNA* and *ZMPSTE24*	Variable from early life to adulthood	Several *LMNA* pathogenic variants, including dominant and recessive ones, result in a spectrum of progeroid laminopathies ranging in severity from severe RD-like forms to adult-onset atypical WS; atypical (severe) MADB forms can result from recessive *ZMPSTE24* pathogenic variants
Werner syndrome (WS)	*WRN*: recessive pathogenic variants	Adulthood	Lack of the pubertal growth spurt during early teen years, graying or loss of hair, scleroderma-like skin lesions, characteristic facies, bilateral cataracts, type 2 diabetes mellitus, hypogonadism, skin ulcers, osteoporosis, arteriosclerosis, increased risk of cancer
Wiedemann-Rautenstrauch syndrome	*POLR3A*	Neonatal	Severe prenatal and postnatal growth restriction, facial dysmorphism, generalized lipodystrophy with local fatty tissue accumulations
Cockayne syndrome	CSA *(ERCC8)* CSB *(ERCC6)*	Neonatal/ infancy	Skin and dental abnormalities, subcutaneous fat loss, short stature, vasculopathy, hypogonadism, hearing loss, cataracts, intellectual disability, neurologic disorders
Rothmund-Thompson syndrome	*RECQL4*	Infancy	Diffuse hair loss, skin and dental abnormalities, short stature, osteopenia, hypogonadism, hyperkeratosis, cataracts, tumor predisposition

Adapted from Coppedè F. Mutations involved in premature-ageing syndromes. *Appl Clin Genet*. 2021;14:279–295.

treatments and a cure. Additional resources include the National Human Genome Research Institute (www.genome.gov/11007255/), National Center for Biotechnology Information Genereviews (www.ncbi.nlm.nih.gov/books/NBK1121/), National Center for Advancing Translational Sciences (www.rarediseases.info.nih.gov/diseases/7467/progeria) and NORD (rarediseases.org).

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Chapter 112

The Porphyrias

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Porphyrias are metabolic diseases resulting from altered activities of specific enzymes of the heme biosynthetic pathway. These enzymes are most active in bone marrow and liver. **Erythropoietic porphyrias**, in which overproduction of heme pathway intermediates occurs primarily in bone marrow erythroid cells, usually present at birth or in early childhood with *cutaneous photosensitivity,* or in the case of congenital erythropoietic porphyria, even in utero as *nonimmune hydrops*. Erythropoietic protoporphyria is the most common porphyria in children. Most porphyrias are hepatic, with overproduction and initial accumulation of porphyrin precursors or porphyrins in the liver. Activation of hepatic porphyrias is very rare during childhood, reflecting the distinct hepatic regulatory mechanisms for heme biosynthesis that are influenced by pubertal development. Homozygous forms of the hepatic porphyrias may manifest clinically before puberty. Children who are heterozygous for inherited hepatic porphyrias may present with nonspecific and unrelated symptoms, and parents often request advice about long-term prognosis and express concerns about drugs that may exacerbate these conditions.

THE HEME BIOSYNTHETIC PATHWAY

Heme is required for a variety of hemoproteins, such as hemoglobin, myoglobin, respiratory cytochromes, and cytochrome P450 enzymes (CYPs). It is believed that the eight enzymes in the pathway for heme biosynthesis are active in all tissues. Hemoglobin synthesis in erythroid precursor cells accounts for approximately 85% of daily heme synthesis in humans. Hepatocytes account for most of the rest, primarily for synthesis of CYPs, which are especially abundant in the liver endoplasmic reticulum and turn over more rapidly than many other hemoproteins, such as the mitochondrial respiratory cytochromes. Pathway intermediates are the porphyrin precursors δ-**aminolevulinic acid** (ALA, also known as *5-aminolevulinic acid*) and **porphobilinogen (PBG)**, as well as porphyrins (mostly in their reduced forms, known as **porphyrinogens**) (Fig. 112.1). These intermediates do not accumulate in significant amounts or have important physiologic functions under normal conditions.

Altered activity of each enzyme in the pathway has been associated with a specific type of porphyria (Table 112.1). The first enzyme, ALA synthase (ALAS), occurs in two forms. An erythroid-specific form, ALAS2, is deficient in X-linked sideroblastic anemia as a result of pathogenic variants of the *ALAS2* gene on chromosome Xp11.2. Gain-of-function pathogenic variants of *ALAS2* caused by deletions in the last exon cause **X-linked protoporphyria (XLP)**, which is phenotypically identical to erythropoietic protoporphyria.

Regulation of heme synthesis differs in the two major heme-forming tissues. Liver heme biosynthesis is primary controlled by the ubiquitous form of ALAS (ALAS1). Synthesis of ALAS1 in liver is regulated by a "free" heme pool (see Fig. 112.1), which can be augmented by newly synthesized heme or by existing heme released from hemoproteins and destined for breakdown to biliverdin by heme oxygenase.

In comparison, in the hematopoietic system, novel regulatory mechanisms allow the production of the very large amounts of heme needed for hemoglobin synthesis. The response to stimuli for hemoglobin synthesis occurs during cell differentiation, leading to an increase in cell number. Also, unlike the liver, heme has a stimulatory role in hemoglobin formation, and the stimulation of heme synthesis in erythroid cells is accompanied not only by increases in ALAS2 but also by sequential induction of other heme biosynthetic enzymes. Separate

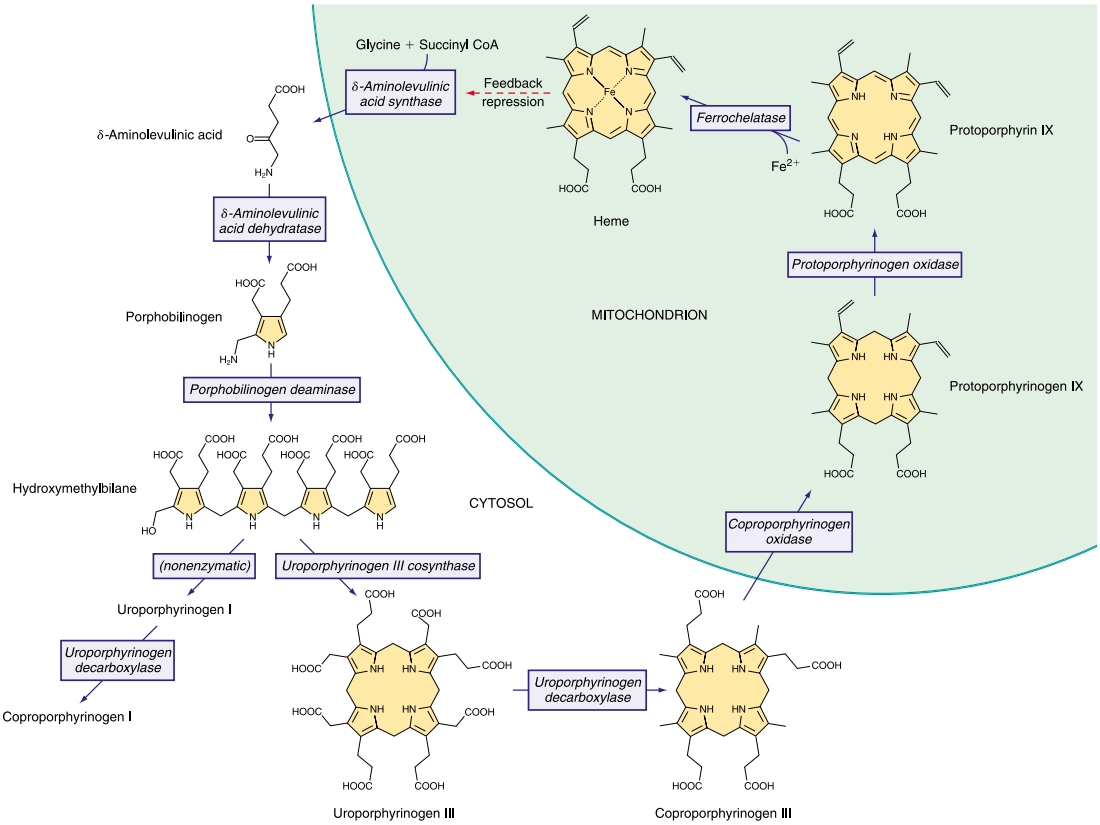

Fig. 112.1 Enzymes and intermediates of the heme biosynthetic pathway. The pathway is regulated in the liver by the end product, heme, mainly by feedback repression *(dashed red arrow)*.

Table 112.1 The Human Porphyrias: Pathogenic Variants, Time of Presentation, and Tissue- and Symptom-Based Classifications

				CLASSIFICATION*			
DISEASE	**ENZYME**	**INHERITANCE**	**PRESENTATION**	**H**	**E**	**A/N**	**C**
X-linked protoporphyria (XLP)	δ-Aminolevulinate synthase 2 (ALAS2)	X-linked	Childhood		X		X
δ-Aminolevulinic acid dehydratase porphyria (ADP)	δ-Aminolevulinic acid dehydratase (ALAD)	Autosomal recessive	Mostly postpuberty	X	X‡	X	
Acute intermittent porphyria (AIP)	Hydroxymethylbilane synthase (HMBS)	Autosomal dominant	Postpuberty	X		X	
Homozygous AIP		Homozygous dominant	Childhood	X	X	X	
Congenital erythropoietic porphyria (CEP)	Uroporphyrinogen III synthase (UROS)	Autosomal recessive	In utero or infancy		X		X
Porphyria cutanea tarda (PCT) type 1	Uroporphyrinogen decarboxylase (UROD)	Sporadic	Adults	X			X
PCT type 2†		Autosomal dominant	Adults	X			X
PCT type 3		Unknown	Adults	X			X
Hepatoerythropoietic porphyria (HEP)		Homozygous dominant	Childhood	X	X‡		X
Hereditary coproporphyria (HCP)	Coproporphyrinogen oxidase (CPOX)	Autosomal dominant	Postpuberty	X		X	X
Homozygous HCP		Homozygous dominant	Childhood	X	X	X	X
Variegate porphyria (VP)	Protoporphyrinogen oxidase (PPOX)	Autosomal dominant	Postpuberty	X		X	X
Homozygous VP		Homozygous dominant	Childhood	X	X	X	X
Erythropoietic protoporphyria (EPP)	Ferrochelatase (FECH)	Autosomal recessive (most commonly heteroallelic with hypomorphic allele)	Childhood		X		X

*Classification abbreviations: H, Hepatic; E, Erythropoietic; A/N, Acute/Neurologic; C, Cutaneous.
†PCT is a result of inhibition of hepatic UROD. Autosomal dominant inheritance of a partial deficiency of UROD is a predisposing factor in cases defined as familial (type 2) PCT. Other genetic factors, such as HFE pathogenic variants, are sometimes found in all types of PCT.
‡ADP and HEP are considered primarily hepatic porphyrias, but substantial increases in erythrocyte zinc protoporphyrin suggest an erythropoietic component.

erythroid-specific and nonerythroid, or "housekeeping," transcripts are known for the first four enzymes in the pathway. The separate forms of ALAS are encoded by genes on different chromosomes, but for each of the other three, erythroid and nonerythroid transcripts are transcribed by alternative promoters in the same gene. Heme also regulates the rate of its synthesis in erythroid cells by controlling the transport of iron into reticulocytes.

Intermediates of the heme biosynthetic pathway are efficiently converted to heme and, normally, only small amounts of the intermediates are excreted. Some may undergo chemical modifications before excretion. Whereas the porphyrin precursors ALA and PBG are colorless, nonfluorescent, and largely excreted unchanged in urine, PBG may degrade to colored products such as the brownish pigment called *porphobilin* or spontaneously polymerize to uroporphyrins. Porphyrins are red in color and display bright-red fluorescence when exposed to long-wavelength ultraviolet (UV) light. Porphyrinogens are the reduced form of porphyrins and are colorless and nonfluorescent, but are readily autoxidized to the corresponding porphyrins when they accumulate or are outside the cell. Only the type III isomers of uroporphyrinogen and coproporphyrinogen are converted to heme (see Fig. 112.1).

ALA and PBG are excreted in urine. Excretion of porphyrins and porphyrinogens in urine or bile is determined by the number of carboxyl groups. Those with many carboxyl groups, such as *uroporphyrin* (octacarboxyl porphyrin) and *heptacarboxyl porphyrin,* are water soluble and readily excreted in urine. Those with fewer carboxyl groups, such as *protoporphyrin* (dicarboxyl porphyrin), are not water soluble and are excreted in bile and feces. *Coproporphyrin* (tetracarboxyl porphyrin) is excreted partly in urine and partly in bile. Because coproporphyrin I is more readily excreted in bile than coproporphyrin III, impaired hepatobiliary function may increase total urinary coproporphyrin excretion and the ratio of these isomers.

CLASSIFICATION AND DIAGNOSIS OF PORPHYRIAS

Two useful classification schemes reflect either the underlying pathophysiology or the clinical features of porphyrias (see Table 112.1). In **hepatic porphyrias** and **erythropoietic porphyrias** the source of excess production of porphyrin precursors and porphyrins is the liver and bone marrow, respectively. **Acute porphyrias** cause neurologic symptoms that are associated with increases of one or both of the porphyrin precursors, ALA and PBG. In the **cutaneous porphyrias,** photosensitivity results from transport of porphyrins in blood from the liver or bone marrow to the skin. **Dual porphyria** refers to the very rare cases of porphyria with deficiencies of two different heme pathway enzymes.

Porphyria cutanea tarda (PCT), acute intermittent porphyria (AIP), and erythropoietic protoporphyria (EPP) are the three most common porphyrias, in that order, considering all age-groups, and are very different in clinical presentation, precipitating factors, methods of diagnosis, and effective therapy (Table 112.2). Two less common acute porphyrias, **hereditary coproporphyria (HCP) and variegate porphyria (VP)**, can also cause blistering photosensitivity (see Table 112.1). **Congenital erythropoietic porphyria (CEP)** causes more severe blistering lesions, often with secondary infection and mutilation. EPP and **X-linked protoporphyria (XLP)** have the same phenotype and are distinct from the other cutaneous porphyrias in causing

Table 112.2 The Three Most Common Human Porphyrias and Major Features

	PRESENTING SYMPTOMS	EXACERBATING FACTORS	MOST IMPORTANT SCREENING TESTS	TREATMENT
Acute intermittent porphyria	Neurologic, adult onset	Drugs (mostly P450 inducers), progesterone, dietary restriction	Urinary porphobilinogen	Hemin, glucose, givosiran
Porphyria cutanea tarda	Skin blistering and fragility (chronic), adult onset	Iron, alcohol, smoking, estrogens, hepatitis C, HIV, halogenated hydrocarbons	Plasma or urine porphyrins	Phlebotomy, low-dose hydroxychloroquine, direct acting antivirals (if hepatitis C is present)
Erythropoietic protoporphyria	Phototoxic pain and swelling (mostly acute), childhood onset	Sunlight exposure	Total erythrocyte protoporphyrin with metal-free and zinc protoporphyrin	Sun protection

nonblistering photosensitivity that occurs acutely after sun exposure. EPP is also the most common porphyria to become manifest before puberty.

First-Line Laboratory Diagnostic Testing

A few sensitive and specific first-line laboratory tests should be obtained whenever symptoms or signs suggest the diagnosis of porphyria. If a first-line or screening test is significantly abnormal, more comprehensive testing should follow to establish the type of porphyria. Overuse of laboratory tests for screening can lead to unnecessary expense and even delay in diagnosis. In patients who present with a past diagnosis of porphyria, laboratory reports that were the basis for the original diagnosis must be reviewed, and if these were inadequate, further testing considered.

Acute porphyria should be suspected in patients with neurovisceral symptoms such as abdominal pain after puberty, when initial clinical evaluation does not suggest another cause. *Urinary PBG* should be measured. Urinary PBG is virtually always increased during acute attacks of AIP, HCP, and VP and is not substantially increased in any other medical conditions. Therefore this measurement is both sensitive and specific. Results from spot (single-void) urine specimens are highly informative because very substantial increases are expected during acute attacks of porphyria. A 24-hour collection can unnecessarily delay diagnosis. The same spot urine specimen should be saved for quantitative determination of PBG (expressed relative to creatinine) to confirm the qualitative PBG result. ALA is often measured as well, but is usually less elevated than PBG in AIP, HCP, and VP. In **ALA dehydratase porphyria (ADP)**, urinary ALA and porphyrins, but not PBG, are greatly elevated. Measurement of urinary porphyrins in addition to PBG is recommended to screen for acute porphyrias because PBG is often less elevated and returns to normal more rapidly in HCP and VP than in AIP. Porphyrin measurement *alone* should be avoided for screening, however, because it is often increased in many disorders other than porphyrias, such as liver diseases, and misdiagnoses of porphyria can result from increases in urinary porphyrins that have no diagnostic significance.

Blistering Cutaneous Porphyrias

Blistering skin lesions caused by porphyria are virtually always accompanied by increases in *total plasma* and *urinary porphyrins.* Porphyrins in plasma in VP are mostly covalently linked to plasma proteins and readily detected by a diagnostic peak in a fluorescence scanning method. The normal range for plasma porphyrins is somewhat increased in patients with end-stage renal disease.

Nonblistering Cutaneous Porphyria

Measurement of total erythrocyte protoporphyrin and, if the total amount is elevated, fractionation of protoporphyrin into its metal-free and zinc-chelated forms, is essential for diagnosis of EPP and XLP. Unfortunately, this is not offered by some major commercial laboratories. Results of zinc protoporphyrin measurements are often recorded (even in the same report) as both *protoporphyrin* and *free erythrocyte protoporphyrin,* with each calculated differently, based on past practices for screening for lead poisoning (which only increases zinc protoporphyrin). Thus the obsolete term *free protoporphyrin* does not mean metal-free protoporphyrin, because it was defined as iron-free protoporphyrin and dates from before it was known that (except in protoporphyrias) protoporphyrin in erythrocytes is mostly zinc chelated. This unnecessary confusion makes diagnosis and reliable exclusion of protoporphyrias difficult. Total plasma porphyrins are elevated in most, but not all, cases of protoporphyria, so a normal level should not be relied on to exclude protoporphyria when total erythrocyte protoporphyrin is elevated.

Increases in erythrocyte total and zinc-chelated protoporphyrin occur in many other conditions, including iron deficiency, lead poisoning, hemolysis, anemia of chronic disease, and other erythrocyte disorders. Therefore the diagnosis of EPP must be confirmed by showing a predominant increase in free and metal-free protoporphyrin. In XLP, both free and zinc protoporphyrin are elevated.

Second-Line Testing

More extensive testing is well justified when a first-line test is positive. A substantial increase in PBG may be caused by AIP, HCP, or VP, and these can be distinguished by measuring urinary porphyrins (using the same spot urine sample), fecal porphyrins, and plasma porphyrins. The various porphyrias that cause blistering skin lesions are differentiated by measuring porphyrins in urine, feces, erythrocytes, and plasma. *Confirmation by genetic testing is important once the diagnosis is established by biochemical testing.*

Testing for Subclinical Porphyria

It is often difficult to diagnose or rule out porphyria in patients who had suggestive symptoms months or years in the past and in asymptomatic relatives of patients with acute porphyrias because porphyrin precursors and porphyrins may be normal. More extensive testing and consultation with a specialist laboratory and physician may be needed. Before evaluating relatives, the diagnosis of porphyria should be firmly established in an index case and the laboratory results reviewed to guide the choice of tests for the family members. Identification of a disease-causing pathogenic variant in an index case greatly facilitates detection of additional gene carriers because biochemical tests in latent carriers may be normal.

δ-AMINOLEVULINIC ACID DEHYDRATASE–DEFICIENT PORPHYRIA

ALA dehydratase–deficient porphyria (ADP) is sometimes termed *Doss porphyria* after the investigator who described the first cases. The term *plumboporphyria* emphasizes the similarity of this condition to lead poisoning, but incorrectly implies that it is caused by lead exposure.

Etiology

This porphyria results from a deficiency of ALA dehydratase (ALAD), which is inherited as an autosomal recessive trait. Only eight cases have been confirmed by pathogenic variant analysis. The prevalence of heterozygous ALAD deficiency was estimated to be <1% in Germany and approximately 2% in Sweden.

Pathology and Pathogenesis

ALAD catalyzes the condensation of two molecules of ALA to form the pyrrole PBG (see Fig. 112.1). The enzyme is subject to inhibition by a number of exogenous and endogenous chemicals. ALAD is the principal lead-binding protein in erythrocytes, and lead can displace the zinc atoms of the enzyme. Inhibition of erythrocyte ALAD activity is a sensitive index of lead exposure.

Eleven abnormal ALAD alleles, most with point pathogenic variants, have been identified, some expressing partial activity, such that heme synthesis is partially preserved. The amount of residual enzyme activity may predict the phenotypic severity of this disease.

ADP is often classified as a hepatic porphyria, although the site of overproduction of ALA is not established. A patient with severe, early-onset disease underwent liver transplantation without significant clinical or biochemical improvement, which might suggest that the excess intermediates did not originate in the liver. Evidence suggests this disease may have an erythropoietic component. Excess urinary coproporphyrin III in ADP might originate from metabolism of ALA to porphyrinogens in a tissue other than the site of ALA overproduction. Administration of large doses of ALA to normal individuals also leads to substantial coproporphyrinuria. Increased erythrocyte zinc protoporphyrin, as in all other homozygous porphyrias, may be explained by accumulation of earlier pathway intermediates in bone marrow erythroid cells during hemoglobin synthesis, followed by their transformation to protoporphyrin after hemoglobin synthesis is complete. Neurologic symptoms are attributed to neurotoxic effects of ALA, but this is unproven.

Clinical Manifestations

In most cases, symptoms resemble other acute porphyrias, including acute attacks of abdominal pain and peripheral neuropathy. Precipitating factors, such as exposure to harmful drugs, have not been evident in most cases. Six of the reported cases were adolescent males. A Swedish infant had more severe disease, with neurologic impairment and failure to thrive. A 63-year-old man in Belgium developed an acute motor polyneuropathy concurrently with a myeloproliferative disorder. A Dutch patient with symptom onset in infancy presented in adulthood with acute symptoms and progressive asymmetric weakness of both extremities.

Laboratory Findings

Urinary ALA, coproporphyrin III, and erythrocyte zinc protoporphyrin are substantially increased. Urinary PBG is normal or slightly increased. Erythrocyte ALAD activity is markedly reduced, and both parents have approximately half-normal activity of this enzyme and normal urinary ALA.

Diagnosis and Differential Diagnosis

The other three acute porphyrias are characterized by substantial increases in both ALA and PBG. In contrast, ALA, but not PBG, is substantially increased in ADP. A marked deficiency of erythrocyte ALAD and half-normal activity in the parents support the diagnosis. Other causes of ALAD deficiency, such as **lead poisoning**, must be excluded. Succinylacetone accumulates in hereditary tyrosinemia type 1 and is structurally similar to ALA, inhibits ALAD, and can cause increased urinary excretion of ALA and clinical manifestations that resemble acute porphyria. Idiopathic acquired ALAD deficiency has been reported. Unlike lead poisoning, the deficient ALAD activity in ADP is not restored by the in vitro addition of sulfhydryl reagents such as dithiothreitol. Even if no other cause of ALAD deficiency is found, it is essential to confirm the diagnosis of ADP by molecular studies.

Treatment

Treatment experience with ADP is limited but is similar to other acute porphyrias. Glucose seems to have minimal effectiveness but may be tried for mild symptoms. **Hemin therapy** was apparently effective for acute attacks in male adolescents, and weekly infusions prevented attacks in two of these patients. Hemin was not effective either biochemically or clinically in the Swedish child with severe disease, and it produced a biochemical response but no clinical improvement in the Belgian man with a late-onset form, who had a peripheral neuropathy but no acute attacks. Hemin is also effective in treating porphyria-like symptoms associated with hereditary tyrosinemia and can significantly reduce urinary ALA and coproporphyrin in lead poisoning. Avoidance of drugs that are harmful in other acute porphyrias is advisable. Liver transplantation was not effective in the child with severe disease. In a recent report, weekly blood transfusions and hydroxycarbamide were used in addition to heme-arginate to suppress erythroid heme synthesis.

Prognosis

Recurrent attacks may occur and require repeated treatment with hemin. The course was unfavorable in the Swedish child with more severe disease and was uncertain in adults with late-onset disease associated with myeloproliferative disorders.

Prevention and Genetic Counseling

Heterozygous parents should be aware that subsequent children are at risk for ADP, as in any autosomal recessive disorder. Prenatal diagnosis is possible but has not been reported.

ACUTE INTERMITTENT PORPHYRIA

AIP is also termed *pyrroloporphyria, Swedish porphyria,* and *intermittent acute porphyria* and is the most common type of acute porphyria in most countries.

Etiology

AIP results from the deficient activity of the housekeeping form of porphobilinogen deaminase (PBGD). This enzyme is also known as **hydroxymethylbilane (HMB) synthase** (the prior term, uroporphyrinogen I synthase, is obsolete). HMB synthase catalyzes the deamination and head-to-tail condensation of four PBG molecules to form the linear tetrapyrrole, HMB (also known as *preuroporphyrinogen;* see Fig. 112.1). A unique dipyrromethane cofactor binds the pyrrole intermediates at the catalytic site until six pyrroles (including the dipyrrole cofactor) are assembled in a linear fashion, after which the tetrapyrrole HMB is released. The apo-deaminase generates the dipyrrole cofactor to form the holodeaminase, and this occurs more readily from HMB than from PBG. Indeed, high concentrations of PBG may inhibit formation of the holodeaminase. The product HMB can cyclize nonenzymatically to form nonphysiologic uroporphyrinogen I, but in the presence of the next enzyme in the pathway is more rapidly cyclized to form uroporphyrinogen III.

Erythroid and housekeeping forms of the enzyme are encoded by a single gene. The two isoenzymes are both monomeric proteins, differ only slightly in molecular weight (approximately 40 and 42 kDa), and result from alternative splicing of two distinct messenger RNA (mRNA) transcripts that arise from two different promoters. The housekeeping promoter functions in all cell types, including erythroid cells.

The pattern of inheritance of AIP is autosomal dominant, with very rare homozygous cases that present in childhood. More than 400 *HMBS* pathogenic variants, including missense, nonsense, and splicing pathogenic variants, and insertions and deletions have been identified in AIP and in many population groups. Most pathogenic variants are found in only one or a few families. Because of founder effects, some are more common in certain geographic areas, such as northern Sweden (W198X), Holland (R116W), Argentina (G116R), Nova Scotia (R173W), and Switzerland (W283X). De novo pathogenic variants may be found in approximately 3% of cases. The nature of the *HMBS* pathogenic variant does not account for the severity of the clinical presentation, which varies greatly within families. **Chester porphyria** was initially described as a variant form of acute porphyria in a large English family but was found to be caused by an *HMBS* pathogenic variant.

Most pathogenic variants lead to approximately half-normal activity of the housekeeping and erythroid isozymes and half-normal amounts of their respective enzyme proteins in all tissues of heterozygotes. In approximately 5% of unrelated AIP patients, the housekeeping isozyme is deficient, but the erythroid-specific isozyme is normal. Pathogenic

variants causing this are usually found within exon 1 or its 5′ splice donor site or initiation of translation codon.

Pathology and Pathogenesis

Induction of the rate-limiting hepatic enzyme ALAS1 is thought to underlie acute exacerbations of this and the other acute porphyrias. AIP remains latent (or asymptomatic) in the great majority of those who are heterozygous carriers of *HMBS* pathogenic variants, and this is almost always the case before puberty. In those with no history of acute symptoms, porphyrin precursor excretion is usually normal, suggesting that half-normal hepatic HMBS activity is sufficient unless hepatic ALAS1 activity is increased. Patients can also be asymptomatic with elevated levels of porphyrin precursors and are classified as *asymptomatic high excretors.* These patients may have a remote history of symptoms. Many factors that lead to clinical expression of AIP, including certain drugs and steroid hormones, have the capacity to induce hepatic ALAS1 and CYPs. When hepatic heme synthesis is increased, half-normal HMBS activity may become limiting, and ALA, PBG, and other heme pathway intermediates may accumulate. In addition, heme synthesis becomes impaired, and heme-mediated repression of hepatic ALAS1 is less effective.

It is not proved, however, that hepatic HMBS remains constant at approximately 50% of normal activity during exacerbations and remission of AIP, as in erythrocytes. An early report suggested that the enzyme activity is considerably less than half-normal in the liver during an acute attack. Hepatic HMBS activity might be reduced further once AIP becomes activated if, as suggested, excess PBG interferes with assembly of the dipyrromethane cofactor for this enzyme. It also seems likely that currently unknown genetic factors play a contributing role in, for example, patients who continue to have attacks even when known precipitants are avoided.

AIP is almost always latent before puberty and becomes active mostly in adult females, which suggests that endocrine factors, and especially adult levels of female steroid hormones, may be important for clinical expression. Premenstrual attacks are probably the result of endogenous progesterone. Acute porphyrias are sometimes exacerbated by exogenous steroids, including oral contraceptive preparations containing progestins. Surprisingly, pregnancy is usually well tolerated, suggesting that beneficial metabolic changes may ameliorate the effects of high levels of progesterone.

Drugs that are unsafe in acute porphyrias (Table 112.3) include those with the capacity to induce hepatic ALAS1, which is closely associated with induction of CYPs. Some chemicals (e.g., griseofulvin) can increase heme turnover by promoting the destruction of specific CYPs to form an inhibitor (e.g., *N*-methyl protoporphyrin) of ferrochelatase (FECH, the final enzyme in the pathway). Sulfonamide antibiotics are harmful but apparently not inducers of hepatic heme synthesis. Ethanol and other alcohols are inducers of ALAS1 and some CYPs.

Nutritional factors, in particular reduced intake of calories and carbohydrates, as may occur with illness or attempts to lose weight, can increase porphyrin precursor excretion and induce attacks of porphyria. Increased carbohydrate intake may ameliorate attacks. Hepatic ALAS1 is modulated by the peroxisome proliferator-activated receptor-γ coactivator-1α, which is an important link between nutritional status and exacerbations of acute porphyria.

Other factors have been implicated. Chemicals in cigarette smoke, such as polycyclic aromatic hydrocarbons, can induce hepatic CYPs and heme synthesis. A survey of AIP patients found an association between smoking and repeated porphyric attacks. Attacks may result from metabolic stress and impaired nutrition associated with major illness, infection, or surgery. Clinical observations suggest an additive effect of multiple predisposing factors, including drugs, endogenous hormones, nutritional factors, and smoking.

Neurologic Mechanisms

The mechanism of neural damage in acute porphyrias is poorly understood. The most favored hypothesis at present is that one or more heme precursors, or perhaps a derivative, are neurotoxic. Increased ALA in AIP, HCP, VP, ADP, plumbism, and hereditary tyrosinemia type 1, which have similar neurologic manifestations, suggests that this substance or a derivative may be neuropathic. Porphyrins derived from ALA after its uptake into cells may have toxic potential. ALA can also interact with γ-aminobutyric acid (GABA) receptors. Severe AIP greatly improves after allogeneic liver transplantation. This experience and the demonstration that recipients of AIP livers develop porphyria support the hypothesis that heme precursors from the liver cause the neurologic manifestations.

Epidemiology

AIP occurs in all ethnic groups and is the most common acute porphyria, with an estimated prevalence in most countries of 5 in 100,000. In Sweden, prevalence was estimated to be 7.7 in 100,000, including latent cases with normal porphyrin precursors. A much higher prevalence of 60-100 in 100,000 in northern Sweden is the result of a founder effect. The combined prevalence of AIP and VP in Finland is approximately 3.4 in 100,000. Population screening by erythrocyte HMBS activity or DNA analysis revealed a prevalence of 200 heterozygotes per 100,000 people in Finland and 1 in approximately 1,675 (60 in 100,000) in France. Studies using exomic/genomic databases show that the estimated frequency of pathogenic variants in the *HMBS* gene is 0.00056 (56 in 100,000), suggesting that the penetrance of this disorder may be as low as 1% and that carriers of *HMBS* pathogenic variants that can cause AIP are much more common than previously believed. Higher penetrance in some families with AIP suggests a strong role of environmental factors and genetic modifiers.

Clinical Manifestations

Neurovisceral manifestations of acute porphyrias may appear any time after puberty, but rarely before it (Table 112.4). Porphyria attacks are exceedingly rare before the onset of puberty. Affected children are more often males as *opposed to affected adolescents and adults*, who are predominantly females. Reported symptomatic childhood cases often lacked adequate biochemical and molecular confirmation. The prepubertal attacks in males may be explained in part by coexisting medical conditions with the potential to upregulate ALAS1. Abdominal pain was the most common presenting symptom in such cases, but seizures, often preceding the diagnosis, were also common. Other manifestations reported in children include tachycardia, peripheral neuropathy, myalgias, hypertension, irritability, lethargy, and behavioral abnormalities. A population-based study in Sweden indicated that symptoms suggestive of porphyria may occur in heterozygotes during childhood, even, in contrast to adults, when urinary porphyria precursors are not elevated. This study did not compare the frequency of such nonspecific symptoms in a control group of children. Very rare cases of homozygous AIP present differently, with severe neurologic manifestations early in childhood.

Acute attacks in adults are characterized by a constellation of nonspecific symptoms, which may become severe and life threatening. **Abdominal pain** occurs in 85–95% of AIP patients and is usually severe, steady, and poorly localized, but is sometimes cramping, and accompanied by signs of ileus, including abdominal distention and decreased bowel sounds. Nausea, vomiting, and constipation are common, but increased bowel sounds and diarrhea may occur. Bladder dysfunction may cause hesitancy and dysuria. **Tachycardia**, the most common physical sign, occurs in up to 80% of attacks. This is often accompanied by **hypertension**, restlessness, coarse or fine tremors, and excess sweating, which are attributed to sympathetic overactivity and increased catecholamines. Other common manifestations include mental symptoms; pain in the extremities, head, neck, or chest; muscle weakness; and sensory loss. Because all these manifestations are neurologic rather than inflammatory, there is little or no abdominal tenderness, fever, or leukocytosis.

Porphyric neuropathy is primarily motor and appears to result from axonal degeneration rather than demyelinization. Sensory involvement is indicated by neuropathic pain in the extremities, which may be described as muscle or bone pain, and by numbness, paresthesias, and dysesthesias. Paresis may occur early in an attack but is more often a late manifestation in an attack that is not recognized

Table 112.3 Drugs Regarded as Unsafe and Safe in Acute Porphyrias

UNSAFE	SAFE
Barbiturates (all)	Narcotic analgesics
Sulfonamide antibiotics*	Aspirin
Meprobamate* (also mebutamate,* tybutamate*)	Acetaminophen (paracetamol)
Carisoprodol*	Phenothiazines
Glutethimide*	Penicillin and derivatives
Methyprylon	Streptomycin
Ethchlorvynol*	Glucocorticoids
Mephenytoin	Bromides
Phenytoin*	Insulin
Succinimides	Atropine
Carbamazepine*	Cimetidine
Clonazepam‡	Ranitidine†
Primidone*	Acetazolamide
Valproic acid*	Allopurinol
Pyrazolones (aminopyrine, antipyrine)	Amiloride
Griseofulvin*	Bethanidine
Ergots	Bumetanide
Metoclopramide* ‡	Coumarins
Rifampin*	Fluoxetine
Pyrazinamide* ‡	Gabapentin
Diclofenac* ‡	Gentamicin
Fluconazole*	Guanethidine
Oral contraceptives	Ofloxacin
Progesterone and synthetic progestins*	Propranolol
Danazol*	Succinylcholine
Alcohol	Tetracycline
ACEIs (especially enalapril) ‡	
Spironolactone	
CCBs (especially nifedipine) ‡	
Ketoconazole	
Ketamine*	

*Porphyria has been listed as a contraindication, warning, precaution, or adverse effect in U.S. labeling for these drugs. Estrogens are also listed as harmful in porphyria but have been implicated as harmful in acute porphyrias, mostly based only on experience with estrogen-progestin combinations. Although estrogens can exacerbate porphyria cutanea tarda, there is little evidence they are harmful in the acute porphyrias.
†Porphyria has been listed as a precaution in U.S. labeling for this drug. However, this drug is regarded as safe by other sources.
‡These drugs have been classified as probably safe by some sources, but this is controversial, and they should be avoided.
This partial listing does not include all available information about drug safety in acute porphyrias. Other sources should be consulted for drugs not listed here.
ACEIs, Angiotensin-converting enzyme inhibitors; CCBs, calcium channel blockers.

and adequately treated. Rarely, severe neuropathy develops when there is little or no abdominal pain. Motor weakness most commonly begins in the proximal muscles of the upper extremities and then progresses to the lower extremities and the periphery. It is usually symmetric, but occasionally asymmetric or focal. Initially, tendon reflexes may be little affected or hyperactive and become decreased or absent. Cranial nerves, most often X and VII, may be affected, and blindness from involvement of the optic nerves or occipital lobes has been reported. More common central nervous system (CNS) manifestations include seizures, anxiety, insomnia, depression, disorientation, hallucinations, and paranoia. Seizures may result from hyponatremia, porphyria itself, or an unrelated cause. Chronic depression and other mental symptoms occur in some patients, but attribution to porphyria is often difficult.

Hyponatremia is common during acute attacks. Inappropriate antidiuretic hormone (ADH) secretion is often the most likely mechanism, but salt depletion from excess renal sodium loss, gastrointestinal (GI) loss, and poor intake have been suggested as causes of hyponatremia in some patients. Unexplained reductions in total blood and red blood cell volumes are sometimes found, and increased ADH secretion might then be an appropriate physiologic response. Other electrolyte abnormalities may include hypomagnesemia and hypercalcemia.

The attack usually resolves within several days unless treatment is delayed. Abdominal pain may resolve within a few hours and paresis within a few days. Even severe motor neuropathy can improve over months or several years but may leave some residual weakness. Progression of neuropathy to respiratory paralysis and death seldom occurs with appropriate treatment and removal of harmful drugs. Sudden death may result from cardiac arrhythmia.

Laboratory Findings

Levels of ALA and PBG are substantially increased during acute attacks. These levels may decrease after an attack but usually remain increased unless the disease becomes asymptomatic for a prolonged period.

Porphyrins are also markedly increased, which accounts for reddish urine in AIP. These are predominantly uroporphyrins, which can form nonenzymatically from PBG. The increased urinary porphyrins in AIP are predominantly isomer III; however, their formation is likely to be largely enzymatic, which might occur if excess ALA produced in the liver enters cells in other tissues and is then converted to porphyrins by the heme biosynthetic pathway. Porphobilin, a degradation product of PBG, and dipyrrolomethanes appear to account for brownish urinary discoloration. Total fecal porphyrins and plasma porphyrins are normal or slightly increased in AIP. Erythrocyte protoporphyrin may be somewhat increased in patients with manifest AIP.

Erythrocyte HMBS activity is approximately half-normal in most patients with AIP. The normal range is wide and overlaps with the range for AIP heterozygotes. Some *HMBS* pathogenic variants cause the enzyme to be deficient only in nonerythroid tissues. HMBS activity is also highly dependent on erythrocyte age, and an increase in erythropoiesis from concurrent illness in an AIP patient may raise the activity into the normal range. Thus measurement of erythrocyte HMBS activity alone is insufficient in testing for AIP.

Diagnosis and Differential Diagnosis

An increased urinary PBG level establishes that a patient has one of the three most common acute porphyrias (see Table 112.2). Measuring PBG in serum is preferred when there is coexistent severe renal disease but is less sensitive when renal function is normal. Measurement of urinary ALA is less sensitive than PBG and also less specific, but will detect ADP, the fourth type of acute porphyria. Measurement of urinary porphyrins is also useful, because they may remain elevated after ALA and PBG return to normal and are also elevated in ADP. Decreased erythrocyte PBGD activity helps confirm the diagnosis of AIP, but is not found in all AIP patients.

Knowledge of the *HMBS* pathogenic variant in a family enables reliable identification of other pathogenic variant carriers. Prenatal diagnosis can be performed by amniocentesis or chorionic villus sampling (CVS) in a fetus with a known *HMBS* pathogenic variant in the family. Prenatal diagnosis is typically not performed because of the low penetrance of the disorder and favorable prognosis with treatment.

Complications

AIP and other acute porphyrias are typically associated with mild abnormalities in liver function tests; some patients develop chronic liver disease. The risk of hepatocellular carcinoma is also increased, perhaps 60- to 70-fold after age 50, even in asymptomatic individuals. Few patients who developed this neoplasm had increases in serum α-fetoprotein. Patients with acute porphyrias, especially >50 years old, should be screened by ultrasound or an alternative imaging method at 6-month intervals.

Table 112.4 Common Presenting Symptoms and Signs of Acute Porphyria

SYMPTOMS AND SIGNS	FREQUENCY (%)	COMMENT
GASTROINTESTINAL		
Abdominal pain	85–95	Usually unremitting (for hours or longer) and poorly localized but can be cramping.
Vomiting	43–88	Neurologic in origin and rarely accompanied by peritoneal signs, fever, or leukocytosis.
Constipation	48–84	Nausea and vomiting often accompany abdominal pain. May be accompanied by bladder paresis.
Diarrhea	5–12	
NEUROLOGIC		
Pain in extremities, back	50–70	Pain may begin in the chest or back and move to the abdomen. Extremity pain from the chest, neck, or head indicates involvement of sensory nerves; objective sensory loss reported in 10–40% of cases.
Paresis	42–68	May occur early or late during a severe attack. Muscle weakness usually begins proximally rather than distally and more often in the upper than lower extremities.
Respiratory paralysis	9–20	Preceded by progressive peripheral motor neuropathy and paresis.
Mental symptoms	40–58	May range from minor behavioral changes to agitation, confusion, hallucinations, and depression.
Convulsions	10–20	A central neurologic manifestation of porphyria or caused by hyponatremia, which often results from syndrome of inappropriate antidiuretic hormone secretion or sodium depletion.
CARDIOVASCULAR		
Tachycardia	64–85	May warrant treatment to control rate, if symptomatic.
Systemic arterial hypertension	36–55	May require treatment during acute attacks, and sometimes becomes chronic.

From Anderson KE, Bloomer JR, Bonkovsky HL, et al. Desnick recommendations for the diagnosis and treatment of the acute porphyrias, *Ann Intern Med.* 2005;142(6):439–450.

The risk of chronic hypertension and impaired renal function is increased in these patients, most often with evidence of interstitial nephritis. A nephrotoxic effect of ALA may contribute. This may progress to severe renal failure and require renal transplantation.

Patients with recurrent attacks may develop **chronic neuropathic pain**, although this has not been well characterized. Referral to a neurologist is recommended for any patient with ongoing or residual neurologic symptoms. In addition, depression and anxiety are common in these patients.

Treatment

Hemin

Intravenous (IV) hemin is the treatment of choice for most acute attacks of porphyria. There is a favorable biochemical and clinical response to early treatment with hemin, but less rapid clinical improvement if treatment is delayed. It is no longer recommended that therapy with hemin for a severe attack be started only after an unsuccessful trial of IV glucose for several days. Mild attacks without severe manifestations, such as paresis, seizures, hyponatremia, or pain requiring opioids, may be treated with IV glucose. After IV administration, hemin binds to hemopexin and albumin in plasma and is taken up primarily in hepatocytes, where it augments the regulatory heme pool in hepatocytes, represses the synthesis of hepatic ALAS1, and dramatically reduces porphyrin precursor overproduction.

Hemin* is available for IV administration in the United States as *lyophilized hematin* (Panhematin, Recordati). Degradation products begin to form as soon as the lyophilized product is reconstituted with sterile water, and these can cause infusion site phlebitis and a transient anticoagulant effect. Repeated treatment can lead to loss of venous access and iron overload. Reconstitution with 30% human albumin can enhance stability and prevent these adverse effects and is recommended especially if a peripheral vein is used for the infusion. Uncommon side effects of hemin include fever, aching, malaise, hemolysis, anaphylaxis, and circulatory collapse. Heme arginate, a more stable hemin preparation, is available in Europe and South Africa.

Hemin treatment should be instituted only after a diagnosis of acute porphyria has been initially confirmed by a marked increase in urinary PBG. When prior documentation of the diagnosis is available for review, it is not essential to confirm an increase in PBG with every recurrent attack if other causes of the symptoms are excluded clinically. The standard regimen of hemin for treatment of acute porphyric attacks is 3-4 mg/kg/day for 4 days. Lower doses have less effect on porphyrin precursor excretion and probably less clinical benefit.

Givosiran, an ALAS1-directed interfering RNA therapeutic, is effective for preventing frequent attacks in adults with acute hepatic porphyrias. Clinical trials have demonstrated rapid lowering of ALA and PBG, resulting in a reduction in attacks, chronic symptoms, and hemin utilization.

General and Supportive Measures

Drugs that may exacerbate porphyrias (see Table 112.3) should be discontinued whenever possible, and other precipitating factors identified. Hospitalization is warranted, except for mild attacks; for treatment of severe pain, nausea, and vomiting; for administration of hemin and fluids; and for monitoring vital capacity, nutritional status, neurologic function, and electrolytes. Pain usually requires an opioid; there is low risk for addiction after recovery from the acute attack. Ondansetron or promethazine is needed for nausea and vomiting and short-acting benzodiazepines for anxiety and restlessness. β-Adrenergic blocking agents may be useful to control tachycardia and hypertension but may be hazardous in patients with hypovolemia and incipient cardiac failure.

Carbohydrate Loading

The effects of carbohydrates on repressing hepatic ALAS1 and reducing porphyrin precursor excretion are weak compared with those of hemin. Therefore carbohydrate loading is recommended only for

* Hemin is the generic name for all heme preparations used for IV administration. Hemin is also a chemical term that refers to the oxidized (ferric) form of heme (iron protoporphyrin IX) and is usually isolated as hemin chloride. In alkaline solution, the chloride is replaced by the hydroxyl ion, forming hydroxyheme, or hematin.

mild attacks (e.g., without severe nausea or vomiting, hyponatremia, seizures, paresis, or pain requiring opioids). Glucose polymer solutions by mouth are sometimes tolerated. At least 300 g of IV glucose, usually given as a 10% solution, has been recommended for adults hospitalized with attacks of porphyria. Amounts up to 500 g daily may be more effective, but large volumes may favor the development of hyponatremia.

Other Therapies

Liver transplantation is effective in patients with severe AIP who are refractory to pharmacologic therapy. Patients can generally expect a complete biochemical and symptomatic resolution after transplantation. However, liver transplantation is a high-risk procedure and should be considered only as a last resort.

Seizures and Other Complications

Patients who experience seizures during an acute attack, especially if caused by hyponatremia or other electrolyte imbalances, may not require prolonged treatment with anticonvulsant drugs, most of which have at least some potential for exacerbating acute porphyrias. Gabapentin, pregabalin, levetiracetam, and vigabatrin are considered safe or probably safe, and clonazepam is probably less harmful than phenytoin, barbiturates, or valproic acid.

Control of hypertension is important and may help prevent chronic renal impairment, which can progress and require renal transplantation.

Safe and Unsafe Drugs

Patients often do well with avoidance of harmful drugs. Listings are available from the **European Porphyria Network** (www.porphyria-europe.com) and **American Porphyria Foundation** (https://porphyriafoundation.org/drugdatabase/), but some listings are controversial. Information regarding safety is lacking for many drugs, especially for those recently introduced.

Exogenous progestins can induce attacks of porphyria. Estrogens are seldom reported to be harmful when given alone. Synthetic steroids with an ethynyl substituent can cause a mechanism-based destruction of hepatic CYPs and should probably be avoided in patients with acute porphyria. Danazol is especially contraindicated.

Other Situations

Major surgery can be carried out safely in patients with acute porphyria, especially if barbiturates are avoided. Halothane has been recommended as an inhalation agent and propofol and midazolam as IV induction agents.

Pregnancy is usually well tolerated, which is surprising, because levels of progesterone, a potent inducer of hepatic ALAS1, are considerably increased during pregnancy. Some females do experience continuing attacks during pregnancy. This has sometimes been attributed to reduced caloric intake or metoclopramide, a drug sometimes used to treat hyperemesis gravidarum and considered by some to be harmful in acute porphyrias.

Diabetes mellitus and other endocrine conditions are not known to precipitate attacks of porphyria. In fact, the onset of diabetes mellitus and resulting high circulating glucose levels may decrease the frequency of attacks and lower porphyrin precursor levels in AIP.

Prognosis

In Finland, 74% of patients with AIP or VP reported that they led normal lives, and <30% had recurrent attacks during several years of follow-up. In those presenting with acute symptoms, recurrent attacks were most likely within the next 1-3 years. Moreover, only 6% of gene carriers who had never had attacks developed symptoms. The positive outlook may result from earlier detection, better treatment of acute attacks, and replacement of harmful drugs such as barbiturates and sulfonamides with safer drugs. However, some patients continue to have recurrent attacks, chronic pain, and other symptoms, even after avoiding known exacerbating factors.

Prevention

For prevention of attacks, it is important to identify multiple inciting factors and remove as many as possible. Drugs for concurrent medical conditions should be reviewed. Because dietary factors are often unapparent, consultation with a dietitian may be useful. A well-balanced diet that is somewhat high in carbohydrate (60–70% of total calories) and sufficient to maintain weight is recommended. There is little evidence that additional dietary carbohydrate helps further in preventing attacks, and it may lead to weight gain. Patients who wish to lose excess weight should do so gradually and when they are clinically stable. Rapid weight loss after bariatric surgery may exacerbate acute porphyrias. Iron deficiency, which can be detected by a low serum ferritin level, should be corrected.

Gonadotropin-releasing hormone (GnRH) analogs, which reversibly suppress ovulation, can be dramatically effective for preventing frequently recurring luteal-phase attacks, but baseline and continuing gynecologic evaluation and bone mineral density measurements are important; transdermal estrogen or a bisphosphonate may be added to prevent bone loss. Hemin administered once or twice weekly can prevent frequent attacks of porphyria in some patients. Alternatively, single-dose hemin can be administered "on demand" at an outpatient infusion center to abort an attack and prevent hospitalization, if a patient can recognize early "prodromal" symptoms.

Givosiran (Givlaari, Alnylam) an interfering RNA therapeutic that is administered subcutaneously every month, has been approved by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) for prevention of acute attacks of an AHP.

Genetic Counseling

A pathogenic variant identified in the index case can be sought in offspring at any age. Counseling should emphasize that the great majority of those who inherit an *HMBS* pathogenic variant never develop symptoms, and the prognosis of those who do is favorable. Therefore a normal, healthy life is expected, especially with avoidance of harmful drugs and other factors and prompt recognition and treatment of symptoms should they occur. Given this favorable outlook, even during pregnancy, having children is not precluded for those who have inherited a pathogenic variant, and prenatal diagnosis of acute porphyrias is less important than it is for many other inherited diseases.

CONGENITAL ERYTHROPOIETIC PORPHYRIA

Also termed *Günther disease,* this rare disease usually presents with photosensitivity shortly after birth or in utero as nonimmune hydrops.

Etiology

CEP is an autosomal recessive disease caused by a marked deficiency of uroporphyrinogen III synthase (UROS). Many *UROS* pathogenic variants have been identified among CEP families. Later-onset disease in adults is often less severe and likely to be associated with myeloproliferative disorders and expansion of a clone of erythroblasts that carry a *UROS* pathogenic variant.

Pathology and Pathogenesis

UROS, which is extremely deficient in CEP, catalyzes inversion of pyrrole ring D of HMB and rapid cyclization of the linear tetrapyrrole to form uroporphyrinogen III. This enzyme is also termed *uroporphyrinogen III cosynthase.* The human enzyme is a monomer. The gene for the enzyme is found on chromosome 10q25.3→q26.3 and contains 10 exons. Erythroid and housekeeping transcripts are generated by alternative promoters but encode the same enzyme.

In CEP, HMB accumulates in erythroid cells during hemoglobin synthesis and cyclizes nonenzymatically to form uroporphyrinogen I, which is auto-oxidized to uroporphyrin I. Some of the uroporphyrinogen I that accumulates is metabolized to coproporphyrinogen I, which accumulates because it is not a substrate for coproporphyrinogen oxidase. Thus both uroporphyrin I and coproporphyrin I accumulate in the bone marrow and are then found in circulating erythrocytes, plasma, urine, and feces.

A variety of *UROS* pathogenic variants have been identified in CEP, including missense and nonsense pathogenic variants, large and small deletions and insertions, splicing defects, and intronic branch-point pathogenic variants. At least four pathogenic variants have been identified in the erythroid-specific promoter. Many patients inherited a different pathogenic variant from each parent, and most pathogenic variants have been detected in only one or a few families. An exception is a common pathogenic variant, C73R, which is at a pathogenic variant hot spot and was found in approximately 33% of alleles. One child with CEP had a *GATA1* pathogenic variant, with no *UROS* pathogenic variant. The CEP phenotype may be modulated by gain-of-function *ALAS2* pathogenic variants, which were first identified as causing XLP.

Genotype-phenotype correlations have been based on the in vitro expression of various CEP pathogenic variants and the severity of associated phenotypic manifestations. The C73R allele, which is associated with a severe phenotype in homozygotes or in patients heteroallelic for C73R and another pathogenic variant expressing little residual activity, resulted in <1% of normal enzyme activity. Patients with the C73R allele and heteroallelic for other pathogenic variants expressing more residual activity have milder disease.

Hemolysis is a common feature of CEP. Excess porphyrins in circulating erythrocytes cause cell damage, perhaps by a phototoxic mechanism, leading to both intravascular hemolysis and increased splenic clearance of erythrocytes. Also important is ineffective erythropoiesis, with intramedullary destruction of porphyrin-laden erythroid cells and breakdown of heme. Expansion of the bone marrow as a result of erythroid hyperplasia may contribute, along with vitamin D deficiency, to bone loss. Nutrient deficiencies sometimes cause erythroid hypoplasia. Despite the marked deficiency of UROS, heme production in the bone marrow is increased because of hemolysis and a compensatory increase in hemoglobin production. This occurs, however, at the expense of marked accumulation of HMB, which is converted to porphyrinogens and porphyrins.

Clinical Manifestations

In severe cases, CEP can cause fetal loss or may be recognized in utero as causing intrauterine hemolytic anemia and nonimmune hydrops fetalis. CEP may be associated with neonatal hyperbilirubinemia, and *phototherapy may unintentionally induce severe cutaneous photosensitivity and scarring.*

The most characteristic presentation is reddish urine or pink staining of diapers by urine or meconium shortly after birth (Fig. 112.2). With sun exposure, severe blistering lesions appear on exposed areas of skin on the face and hands and have been termed *hydroa estivale* because they are more severe with greater sunlight exposure during summer (Fig. 112.3). Vesicles and bullae, as well as friability, hypertrichosis, scarring, thickening, and areas of hypopigmentation and hyperpigmentation, are very similar to those seen in PCT but usually much more severe. Infection and scarring sometimes cause loss of facial features and fingers and damage to the cornea, ears, and nails. Porphyrins are deposited in dentin and bone in utero. Reddish-brown teeth in normal light, an appearance termed **erythrodontia**, display reddish fluorescence under long-wave UV light (Fig. 112.4). Unaffected children born to a mother with CEP may have erythrodontia. Hemolysis and splenomegaly are common in CEP. Bone marrow compensation may be adequate, especially in milder cases. Patients with severe phenotypes, however, are often transfusion dependent. Splenomegaly may contribute to the anemia and cause leukopenia and thrombocytopenia, which may be complicated by significant bleeding. Neuropathic symptoms are absent, and there is no sensitivity to drugs, hormones, or carbohydrate restriction. The liver may be damaged by iron overload, viral hepatitis, or other causes.

Milder cases of CEP with onset of symptoms in adult life and without erythrodontia may be misdiagnosed initially as PCT. Late-onset cases are likely to be associated with myeloproliferative disorders and expansion of a clone of erythroid cells carrying a *UROS* pathogenic variant.

Fig. 112.2 Congenital erythropoietic porphyria (CEP). The diaper of a baby with CEP demonstrates the red color of urine. (*From Paller AS, Macini AJ. Hurwitz Clinical Pediatric Dermatology, 3rd ed. Philadelphia: Saunders; 2006: p. 517.*)

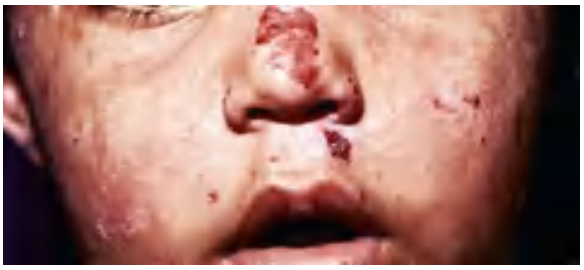

Fig. 112.3 Congenital erythropoietic porphyria. Vesicles, bullae, and crusts on sun-exposed areas. (*From Paller AS, Macini AJ. Hurwitz Clinical Pediatric Dermatology, 3rd ed. Philadelphia: Saunders; 2006: p. 517.*)

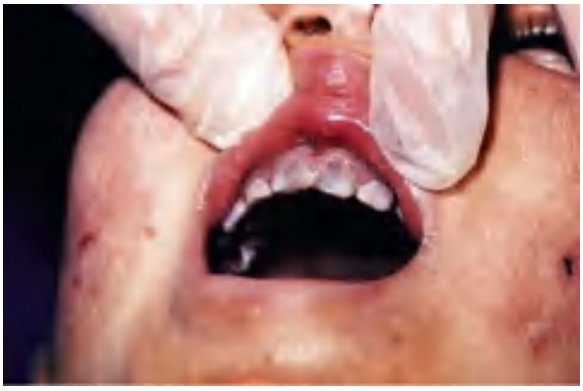

Fig. 112.4 Congenital erythropoietic porphyria. Brownish teeth that fluoresce under Wood's lamp examination. (*From Paller AS, Macini AJ. Hurwitz Clinical Pediatric Dermatology, 3rd ed. Philadelphia: Saunders; 2006: p. 517.*)

Laboratory Findings

Urinary porphyrin excretion and circulating porphyrin levels in CEP are much higher than in almost all other porphyrias. Urinary porphyrin excretion can be as high as 50-100 mg daily and consists mostly of uroporphyrin I and coproporphyrin I. ALA and PBG are normal. Fecal porphyrins are greatly increased, with a predominance of coproporphyrin I.

Marked increases in erythrocyte porphyrins in CEP also consist mostly of uroporphyrin I and coproporphyrin I. These porphyrins are also increased in bone marrow, spleen, plasma, and to a lesser extent, liver. The porphyrin pattern in erythrocytes is influenced by rates of erythropoiesis and erythroid maturation. A predominance of protoporphyrin has been noted in some CEP patients, and in one such patient, uroporphyrin and coproporphyrin increased when erythropoiesis was stimulated by blood removal.

Diagnosis and Differential Diagnosis

The diagnosis of CEP should be documented by full characterization of porphyrin patterns and identification of the underlying pathogenic variants. In later-onset cases, an underlying myeloproliferative disorder and a *UROS* somatic pathogenic variant should be suspected and studied in detail.

The clinical picture in hepatoerythropoietic porphyria (HEP) may be similar, but the porphyrin patterns in urine and feces in HEP resemble PCT. A predominant increase in erythrocyte protoporphyrin is not expected in CEP, except in mild cases, but is characteristic of HEP and rare homozygous cases of AIP, HCP, and VP. EPP and XLP are also distinguished by normal urinary porphyrins and by increases in erythrocyte metal-free protoporphyrin, whereas the increased protoporphyrin in other conditions is mostly complexed with zinc.

CEP should be suspected as a cause of nonimmune hydrops or hemolytic anemia in utero. With recognition of the disease at this stage, intrauterine transfusion can be considered, and phototherapy for hyperbilirubinemia after birth, which can cause severe blistering and scarring, can be avoided. Prenatal diagnosis is feasible by finding red-brown discoloration and increased porphyrins in amniotic fluid and measuring porphyrins in fetal erythrocytes and plasma. *UROS* pathogenic variants can be identified in chorionic villi or cultured amniotic cells.

Treatment

Advising patients to avoid sunlight exposure is essential, because sunlight exposure does not cause immediate severe pain in CEP, in contrast to the protoporphyrias. Minimizing skin trauma and prompt treatment of any cutaneous infections are also essential. Sunscreen lotions and beta-carotene are of little benefit. Transfusions to achieve a level of hemoglobin sufficient to significantly suppress erythropoiesis can be quite effective in reducing porphyrin levels and photosensitivity. Concurrent deferoxamine to reduce iron overload and hydroxyurea to suppress erythropoiesis further may provide additional benefit. Splenectomy reduces hemolysis and transfusion requirements in some patients. Iron restriction by phlebotomy or iron chelators may improve photosensitivity in CEP patients by decreasing ALAS2 activity and porphyrin production.

The most effective treatment is marrow stem cell transplantation in early childhood, which has greatly reduced porphyrin levels and photosensitivity and increased long-term survival.

Prognosis

The outlook is favorable in milder cases and in patients with more severe disease, especially after successful bone marrow or stem cell transplantation. Otherwise, prognosis relates to adherence to sunlight avoidance.

Prevention and Genetic Counseling

Genetic counseling is important for affected families because CEP can be recognized before birth, and a severe phenotype can often be predicted by identifying the nature of the *UROS* pathogenic variants.

PORPHYRIA CUTANEA TARDA

Porphyria cutanea tarda is the most common and readily treated human porphyria (see Table 112.2). It occurs in mid or late adult life and is rare in children. Previous terms include *symptomatic porphyria, PCT symptomatica,* and *idiosyncratic porphyria.* The underlying cause is a liver-specific acquired deficiency of uroporphyrinogen decarboxylase (UROD) with contributions by genetic and acquired susceptibility factors, including heterozygous *UROD* pathogenic variants in familial PCT. HEP, the homozygous form of familial PCT, usually has a more severe presentation in childhood, resembling CEP clinically.

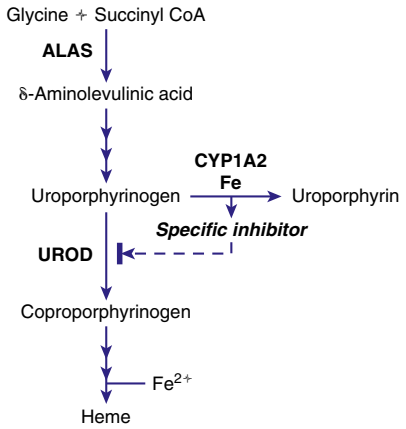

Fig. 112.5 Formation of a specific inhibitor of uroporphyrinogen decarboxylase in the liver in porphyria cutanea tarda. ALAS, δ-Aminolevulinic acid synthase; CYP1A2, cytochrome P450 1A2; UROD, uroporphyrinogen decarboxylase.

Etiology

PCT is caused by a reduction of hepatic UROD activity to ≤20% of normal activity. An inhibitor of hepatic UROD has been characterized as a *uroporphomethene,* which is derived from partial oxidation of the enzyme substrate uroporphyrinogen. CYPs, such as CYP1A2, as well as iron, are involved in its formation (Fig. 112.5). Although enzyme activity is inhibited, the amount of hepatic enzyme protein measured immunochemically remains at its genetically determined level.

UROD catalyzes the decarboxylation of the four acetic acid side chains of uroporphyrinogen (an octacarboxyl porphyrinogen) to form coproporphyrinogen (a tetracarboxyl porphyrinogen). The enzyme reaction occurs in a sequential, clockwise fashion, with the intermediate formation of hepta-, hexa-, and pentacarboxyl porphyrinogens. Uroporphyrinogen III, as compared with other uroporphyrinogen isomers, is the preferred substrate. Human UROD is a dimer with the two active site clefts juxtaposed. The *UROD* gene contains 10 exons, with only one promoter. Therefore the gene is transcribed as a single mRNA in all tissues.

The majority of PCT patients (80%) have no *UROD* pathogenic variants and have sporadic **(type 1)** disease. Some are heterozygous for *UROD* pathogenic variants and have familial **(type 2)** PCT. Described pathogenic variants include missense, nonsense, and splice-site pathogenic variants; several small and large deletions; and small insertions, with only a few identified in more than one family. A few of these pathogenic variants may be located near the active site cleft, but most appear to involve regions with important structural roles. Being heterozygous for a *UROD* pathogenic variant is insufficient to cause PCT. Individuals with type 2 PCT are born with 50% of normal UROD activity, and later in life other susceptibility factors (as in type 1) lead to production of the uroporphomethene inhibitor and further reduction on hepatic UROD activity to <20% of normal. Because penetrance of the genetic trait is low, many patients with familial PCT have no family history of the disease.

Induction of hepatic ALAS1 is not a prominent feature in PCT, although alcohol may increase this enzyme slightly. Iron and estrogens are not potent inducers of ALAS1, and drugs that are potent inducers of ALAS1 and CYPs are much less frequently implicated in PCT than in acute porphyrias.

Blistering skin lesions result from porphyrins that circulate from the liver. Sunlight exposure leads to generation of reactive oxygen species (ROS) in the skin, complement activation, and lysosomal damage.

Epidemiology

Differences in prevalence probably relate to geographic variations in susceptibility factors such as hepatitis C and ethanol use. The yearly incidence in the United Kingdom was estimated at 2-5 in 1 million in the general population, and the prevalence in the United States and Czechoslovakia was estimated at 1 in 25,000 and 1 in 5,000 in the general population, respectively. The disease was reported to be prevalent in the Bantus of South Africa in association with iron overload. PCT is more common in males, possibly because of greater alcohol intake, and in women it is usually associated with estrogen use.

A massive outbreak of PCT occurred in eastern Turkey in the 1950s. Wheat intended for planting and treated with hexachlorobenzene as a fungicide was consumed by many during a food shortage. Cases and small outbreaks of PCT after exposure to other chemicals, including di- and trichlorophenols and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, dioxin), have been reported. The manifestations improved in most cases when the exposure was stopped. There are reported cases of delayed onset many years after chemical exposure.

Pathology and Pathogenesis

Generation of a UROD inhibitor in the liver plays an important role in all three types of PCT. Eighty percent of patients with type 1 **(sporadic)** PCT have no *UROD* pathogenic variants, and UROD activity is normal in nonhepatic tissues such as erythrocytes. In type 2 **(familial)** PCT, a heterozygous *UROD* pathogenic variant results in a partial (approximately 50%) deficiency of UROD in all tissues from birth, and the disease becomes active in some heterozygotes after further reduction of hepatic UROD activity to ≤20% of normal. **Type 3** is rare and describes PCT without a *UROD* pathogenic variant occurring in more than one family member. Another genetic basis, such as *HFE* pathogenic variants, may be identified. HEP results from inheritance of a *UROD* pathogenic variant from each parent and typically causes severe photosensitivity resembling CEP starting in early childhood. Some developed symptoms in childhood more typical of PCT.

CYPs, especially CYP1A2, can catalyze the oxidation of uroporphyrinogen to uroporphyrin. This uroporphyrinogen oxidase activity is enhanced by iron and leads to formation of a UROD inhibitor (see Fig. 112.5). CYP1a2 seems essential for development of uroporphyria in rodents, because experimental uroporphyria does not develop in *CYP1a2* knockout mice.

Susceptibility Factors

The following factors are implicated in the development of PCT, and these occur in various combinations in individual patients.

Iron

PCT is an iron-related disorder, and a normal or increased amount of iron in the liver is essential for its development. Moreover, treatment by phlebotomy to reduce hepatic iron leads to remission. Serum ferritin levels are usually in the upper part of the normal range or moderately increased, and liver histology commonly shows increased iron staining. Prevalence of the C282Y pathogenic variant of the *HFE* gene, which is the major cause of hemochromatosis in people of Northern European ancestry, is increased in both type 1 and type 2 PCT, and approximately 10% of patients are C282Y homozygotes. In Southern Europe the H63D pathogenic variant is more prevalent. PCT may develop in patients with secondary iron overload. Reduced hepatic expression of the hormone hepcidin occurs in hemochromatosis and also in PCT, regardless of *HFE* genotype, which may explain hepatic siderosis in this condition.

Hepatitis C

Hepatitis C virus (HCV) infection is highly prevalent in PCT in most geographic locations; in the United States, for example, HCV is present in 56–74% of cases, which is similar to rates in Southern Europe. Prevalence of hepatitis C in PCT is lower in Northern Europe (<20%). Steatosis and oxidative stress in HCV infection may favor iron-mediated generation of ROS and a UROD inhibitor. Dysregulation of hepcidin occurs in hepatitis C and may lead to increased iron absorption.

Human Immunodeficiency Virus

Many reports suggest that HIV infection can contribute to the development of PCT, although less frequently than HCV.

Ethanol

The long-recognized association between alcohol and PCT may be explained by the generation of ROS, which may cause oxidative damage, mitochondrial injury, depletion of reduced glutathione and other antioxidant defenses, increased production of endotoxin, and activation of Kupffer cells. Also, alcohol may contribute to iron overload by impairing hepcidin production.

Smoking and Cytochrome P450 Enzymes

Although not extensively studied as a susceptibility factor, smoking is often associated with alcohol use in PCT. It may act to induce hepatic CYPs and oxidative stress. Hepatic CYPs are thought to be important in oxidizing uroporphyrinogen and generating a UROD inhibitor (see Fig. 112.5). Genetic polymorphisms of *CYP1A2* and *CYP1A1* have been implicated in human PCT. The frequency of an inducible *CYP1A2* genotype was more common in PCT patients than in controls in several studies.

Antioxidant Status

Ascorbic acid deficiency contributes to uroporphyria in laboratory models and perhaps in human PCT. In one series, plasma ascorbate levels were substantially reduced in 84% of patients with PCT. Low levels of serum carotenoids were also described, further suggesting that oxidant stress in hepatocytes is important in PCT.

Estrogens

Use of estrogen-containing oral contraceptives (OCs) or postmenopausal estrogen replacement therapy is frequently associated with PCT (type 1 or 2) in women. PCT sometimes occurs during pregnancy, although it is not clear whether the risk is increased.

Cancer Chemotherapy

Cancer chemotherapeutic agents are commonly associated with rare childhood cases of PCT, although specific agents have not been implicated. Susceptibility factors found in adults are rare in children with PCT.

Clinical Manifestations

Cutaneous Manifestations

PCT is readily recognized by blistering and crusted skin lesions on the backs of the hands, which are the most sun-exposed areas of the body, and less often on the forearms, face, ears, neck, legs, and feet (Fig. 112.6). The fluid-filled vesicles usually rupture and become crusted or denuded areas, heal slowly, and are subject to infection. The skin is friable, and minor trauma may cause blisters or denudation of skin. Small white plaques, termed *milia,* may precede or follow vesicle formation. Facial hypertrichosis and hyperpigmentation are also common. Severe scarring and thickening of sun-exposed skin may resemble scleroderma. Skin biopsy findings include subepidermal blistering and deposition of periodic acid–Schiff-(PAS)–positive material around blood vessels and fine fibrillar material at the dermoepithelial junction, which may be related to excessive skin fragility. IgG, other immunoglobulins, and complement are also deposited at the dermoepithelial junction and around dermal blood vessels. The skin lesions and histologic changes are not specific for PCT. The same findings occur in VP and HCP and resemble those of CEP and HEP but are usually less severe. PCT usually develops in mid or late adult life. Earlier onset may be seen in those with *UROD* or *HFE* pathogenic variants and childhood onset with cancer chemotherapy and *UROD* pathogenic variants.

Liver Abnormalities

PCT is almost always associated with nonspecific liver abnormalities, especially increased serum transaminases and γ-glutamyltranspeptidase, even in the absence of heavy alcohol intake or hepatitis C. Most histologic findings, such as necrosis, inflammation,

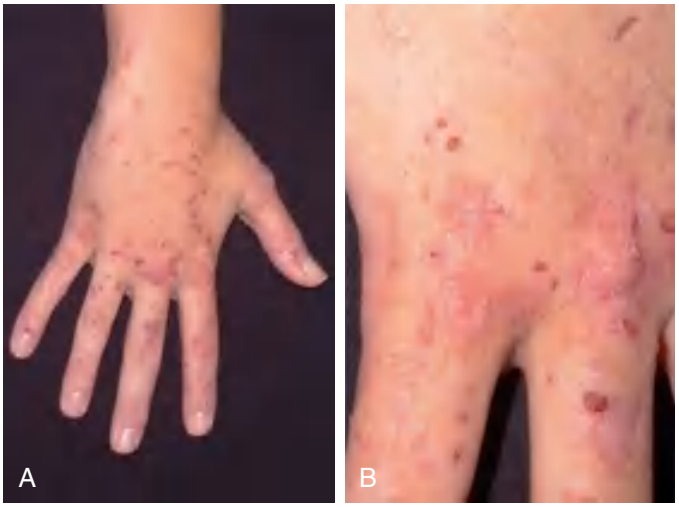

Fig. 112.6 Porphyria cutanea tarda (PCT). A, Right hand of a patient with PCT, revealing numerous erosions and erythematous patches. B, Close-up of right hand. (*From Horner ME, Alikhan A, Tintle S, et al. Cutaneous porphyrias. Part 1. Epidemiology, pathogenesis, presentation, diagnosis, and histopathology. Int J Dermatol. 2013;52:1464–1480, Fig. 2.*)

increased iron, and increased fat, are nonspecific. Specific findings include red fluorescence of liver tissue and fluorescent, birefringent, needle-like inclusions presumably consisting of porphyrins. Electron microscopy shows these inclusions are in lysosomes, and paracrystalline inclusions are found in mitochondria. Distorted lobular architecture and cirrhosis are more common with long-standing disease.

The risk of developing hepatocellular carcinoma is increased, with reported incidences ranging from 4% to 47% in PCT. These tumors seldom contain large amounts of porphyrins.

Other Features and Associations

Mild or moderate erythrocytosis in some adult patients is not well understood, but chronic lung disease from smoking may contribute. An earlier onset of symptoms may be noted in patients with genetic predisposing factors, such as an inherited partial deficiency of UROD or the C282Y/C282Y *HFE* genotype. Iron overload secondary to conditions such as myelofibrosis and end-stage renal disease (ESRD) may be associated with PCT. The disease can be especially severe in patients with ESRD because the lack of urinary excretion leads to much higher concentrations of porphyrins in plasma, and the excess porphyrins are poorly dialyzable. PCT occurs more frequently in patients with systemic lupus erythematosus and other immunologic disorders than would be expected by chance.

Laboratory Findings

Porphyrins accumulate in the liver mostly as the oxidized porphyrins rather than porphyrinogens in PCT, as indicated by the immediate *red fluorescence* observed in liver tissue. This develops over weeks or months before porphyrins appear in plasma and are transported to the skin, causing photosensitivity. In contrast to the acute hepatic porphyrias, only a very small increase in synthesis of heme pathway intermediates and little or no increase in hepatic ALAS1 are required to account for the excess porphyrins excreted in PCT.

Hepatic UROD deficiency leads to a complex pattern of excess porphyrins, which initially accumulate as porphyrinogens and then undergo nonenzymatic oxidation to the corresponding porphyrins (uro-, hepta-, hexa-, and pentacarboxyl porphyrins, and isocoproporphyrins). Uroporphyrin and heptacarboxyl porphyrin predominate in urine, with lesser amounts of coproporphyrin and penta- and hexacarboxyl porphyrin. A normally minor pathway is accentuated by UROD deficiency, whereby pentacarboxyl porphyrinogen is oxidized by coproporphyrinogen oxidase (CPOX; the next enzyme in the pathway), forming isocoproporphyrinogen, an atypical tetracarboxyl porphyrinogen. Relative to normal values, urinary porphyrins are increased to a greater extent than fecal porphyrins. However, the total amount of porphyrins excreted in feces in PCT exceeds that in urine, and total excretion of type III isomers (including isocoproporphyrins, which are mostly derived from the type III series) exceeds that of type I isomers. Perhaps because uroporphyrinogen III is the preferred substrate for UROD, more uroporphyrinogen I than III accumulates and is excreted as uroporphyrin I in PCT. Hepta- and hexacarboxyl porphyrin are mostly isomer III, and pentacarboxyl porphyrin and coproporphyrin are approximately equal mixtures of isomers I and III.

Diagnosis and Differential Diagnosis

Plasma and urine porphyrins are always increased in clinically manifest PCT, and their measurement is useful for screening. A normal value rules out PCT and other porphyrias that produce blistering skin lesions. It is useful to determine the plasma fluorescence emission maximum at neutral pH, because a maximum near 619 nm is characteristic of PCT (as with CEP and HCP) and, importantly, excludes VP, which has a distinctly different fluorescence maximum. Increased urinary or plasma porphyrins, with a predominance of uroporphyrin and heptacarboxyl porphyrin, is characteristic, although not absolutely specific, for PCT and may occasionally be seen in other porphyrias. Nonspecific increases in urine porphyrins, especially of coproporphyrin, occur in liver disease and other medical conditions. Urinary ALA may be increased slightly in PCT, and PBG is normal. Mild cases of CEP can mimic PCT clinically, and this possibility is ruled out by finding normal or only mildly increased levels of erythrocyte porphyrins.

Familial (type 2) can be distinguished from sporadic (type 1) PCT by finding decreased erythrocyte UROD activity (in type 2) or, more reliably, by finding a disease-related *UROD* pathogenic variant. Type 3 is distinguished from type 1 only by occurrence of PCT in a relative. Biochemical findings in HEP are similar to those in PCT, but with an additional marked increase in erythrocyte zinc protoporphyrin.

Pseudoporphyria (also known as *pseudo-PCT*) presents with skin lesions that closely resemble PCT, but without significant increases in plasma or urine porphyrins. A photosensitizing agent such as a nonsteroidal antiinflammatory drug (NSAID) is sometimes implicated. Both PCT and pseudoporphyria may occur in patients with ESRD.

Complications

Cutaneous blisters may rupture and become infected, sometimes leading to cellulitis. In more severe disease in patients with ESRD, repeated infections can be mutilating, as in CEP. **Pseudoscleroderma**, with scarring, contraction, and calcification of skin and subcutaneous tissue, is a rare complication. Other complications include advanced liver disease and hepatocellular carcinoma.

Treatment

Two specific and effective forms of treatment, phlebotomy and low-dose hydroxychloroquine, are available. Susceptibility factors should be removed when possible. The diagnosis of PCT must be firmly established because conditions, including other porphyrias, that produce identical cutaneous lesions do not respond to these treatments. Treatment can usually be started after demonstrating an increase in plasma total porphyrins and excluding VP by analysis of the fluorescence spectrum at neutral pH, while urine and fecal studies are still pending. Use of alcohol, estrogens (in women), and smoking should be stopped, and patients tested for HCV, HIV, and *HFE* pathogenic variants. Susceptibility factors and degree of iron overload, as assessed by the serum ferritin concentration, can influence the choice of treatment.

Phlebotomy is considered standard therapy and is effective in both children and adults with PCT because it reduces hepatic iron content. Treatment is guided by plasma (or serum) ferritin and porphyrin levels. Hemoglobin or hematocrit levels should be followed to prevent symptomatic anemia. For adults, a unit of blood (450 mL) is removed at about 2-week intervals until a target serum ferritin near the lower limit of normal (15 ng/mL) is achieved. A total of six to eight phlebotomies is often sufficient in adults. After this, plasma porphyrin

concentrations continue to fall from pretreatment levels (generally 10-25 μg/dL) to below the upper limit of normal (1 μg/dL), usually after several more weeks. This is followed by gradual clearing of skin lesions, sometimes including pseudoscleroderma. Liver function abnormalities may improve, and hepatic siderosis, needle-like inclusions, and red fluorescence of liver tissue will disappear. Although remission usually persists even if ferritin levels later return to normal, it is advisable to follow porphyrin levels and reinstitute phlebotomies if porphyrins begin to increase. Infusions of deferoxamine, an iron chelator, may be used when phlebotomy is contraindicated.

An alternative when phlebotomy is contraindicated or poorly tolerated is a low-dose regimen of hydroxychloroquine (or chloroquine). Normal doses of these 4-aminoquinoline antimalarials in PCT increase plasma and urinary porphyrin levels and increase photosensitivity, reflecting an outpouring of porphyrins from the liver. This is accompanied by acute hepatocellular damage, with fever, malaise, nausea, and increased serum transaminases, but is followed by complete remission of the porphyria. These adverse consequences of normal doses are largely avoided by a low-dose regimen (for adults, hydroxychloroquine 100 mg or chloroquine 125 mg, i.e., half a normal tablet, twice weekly), which can be continued until plasma or urine porphyrins are normalized. In young children, half the adult dose is recommended. There is at least some risk of retinopathy, which may be lower with hydroxychloroquine. The mechanism of action of 4-aminoquinolines in PCT is not known but is quite specific, because these drugs are not useful in other porphyrias. Recent studies indicate that low-dose hydroxychloroquine is as safe and effective as phlebotomy in adults with PCT.

Experience to date supports treatment of patients with PCT and hepatitis C with direct-acting antiviral agents to achieve cure of this viral infection within a few months as well as remission of PCT and avoiding treatment by phlebotomy or low-dose hydroxychloroquine.

PCT in patients with ESRD is often more severe and difficult to treat. However, erythropoietin administration can correct anemia, mobilize iron, and support phlebotomy in many cases. Improvement is expected after renal transplantation, related in part to resumption of endogenous erythropoietic production.

Liver imaging and a serum α-fetoprotein determination may be advisable in all PCT patients with cirrhosis or advanced fibrosis at 6-month intervals for early detection of hepatocellular carcinoma. Finding low-erythrocyte UROD activity or a *UROD* pathogenic variant identifies those with an underlying genetic predisposition, which does not alter treatment but is useful for genetic counseling.

Prognosis

Porphyria cutanea tarda is the most readily treated form of porphyria, and complete remission is expected with treatment by phlebotomy or low-dose hydroxychloroquine or by treatment of hepatitis C. There is little information on rates of recurrence and long-term outlook. Risk for cirrhosis and hepatocellular carcinoma is increased, and some susceptibility factors such as alcohol, hepatitis C, and iron overload can contribute to such risks.

Prevention and Genetic Counseling

A heritable *UROD* pathogenic variant can usually be detected or excluded by measuring erythrocyte UROD activity, although DNA studies are more sensitive. Relatives of patients with *UROD* pathogenic variants have an increased risk for developing PCT and may have increased motivation to avoid adverse behaviors such as ethanol and tobacco use and exposures to HCV and HIV (although such counseling would be given to anyone). The finding of *HFE* pathogenic variants, and especially C282Y, should prompt screening of relatives, some of whom may be C282Y homozygotes and warrant lifelong monitoring of serum ferritin.

HEPATOERYTHROPOIETIC PORPHYRIA

HEP is the homozygous form of familial (type 2) PCT; it resembles CEP clinically. Excess porphyrins originate mostly from liver, with a pattern consistent with severe UROD deficiency.

Etiology

HEP is an autosomal recessive disorder, and most patients have inherited different pathogenic variants from unrelated parents. In contrast to most pathogenic variants in familial PCT, most causing HEP are associated with expression of some residual enzyme activity. At least one genotype is associated with the predominant excretion of pentacarboxyl porphyrin.

Pathology and Pathogenesis

Excess porphyrins originate primarily from the liver in HEP, although the substantial increase in erythrocyte zinc protoporphyrin indicates that the heme biosynthetic pathway is also impaired in bone marrow erythroid cells. Apparently, porphyrinogens accumulate in the marrow while hemoglobin synthesis is most active and are metabolized to protoporphyrin after hemoglobin synthesis is complete. The cutaneous lesions are a result of photoactivation of porphyrins in skin, as in other cutaneous porphyrias.

Clinical Manifestations

This disease usually presents with blistering skin lesions, hypertrichosis, scarring, and red urine in infancy or childhood. *Sclerodermoid* skin changes are sometimes prominent. Unusually mild cases have been described. Concurrent conditions that affect liver function can alter disease severity; the disease manifested because of hepatitis A in a 2-year-old child and then improved with recovery of liver function.

Laboratory Findings

Biochemical findings resemble those in PCT, with accumulation and excretion of uroporphyrin, heptacarboxyl porphyrin, and isocoproporphyrin. In addition, erythrocyte zinc protoporphyrin is substantially increased.

Diagnosis and Differential Diagnosis

HEP is distinguished from CEP by increases in both uroporphyrin and heptacarboxyl porphyrin and isocoproporphyrins. In CEP, the excess erythrocyte porphyrins are predominantly uroporphyrin I and coproporphyrin I rather than protoporphyrin. Blistering skin lesions are unusual in EPP, the excess erythrocyte protoporphyrin in that disease is metal-free and not complexed with zinc, and urinary porphyrins are normal.

Treatment and Prognosis

Avoiding sunlight exposure is most important in managing HEP, as in CEP. Oral charcoal was helpful in a severe case associated with dyserythropoiesis. Phlebotomy has shown little or no benefit. The outlook depends on the severity of the enzyme deficiency and may be favorable if sunlight can be avoided.

Prevention and Genetic Counseling

As part of genetic counseling in affected families, it is feasible to diagnose HEP in utero, either by analysis of porphyrins in amniotic fluid or DNA studies.

HEREDITARY COPROPORPHYRIA

This autosomal dominant hepatic porphyria is caused by a deficiency of CPOX. The disease presents with acute attacks, as in AIP. Cutaneous photosensitivity may occur, but much less often than in VP. Rare homozygous cases present in childhood, including a variant form known as *harderoporphyria*.

Etiology

A partial (50%) deficiency in CPOX activity has been found in all cells studied from patients with HCP. A much more profound deficiency is found in homozygous cases. Human CPOX is a homodimer composed of 39-kDa subunits and contains no metals or prosthetic groups. The enzyme requires molecular oxygen and is localized in the mitochondrial intermembrane space. A single active site on the enzyme catalyzes the oxidative decarboxylation of two of the four propionic acid groups of coproporphyrinogen III to form the two vinyl groups at positions

two and four, on rings A and B, respectively, of protoporphyrinogen IX. Most of the intermediate tricarboxyl porphyrinogen, termed *harderoporphyrinogen,* is not released before undergoing the second decarboxylation to protoporphyrinogen IX. Coproporphyrinogen I is not a substrate for this enzyme.

The human *CPOX* gene contains a single promoter with elements for both housekeeping and erythroid-specific expression. A variety of *CPOX* pathogenic variants have been described in HCP, with a predominance of missense pathogenic variants and no genotype-phenotype correlations. **Harderoporphyria**, an autosomal recessive biochemical variant form of HCP, is caused by *CPOX* pathogenic variants that impair substrate binding, leading to premature release of harderoporphyrinogen.

Epidemiology

HCP is less common than AIP and VP, but its prevalence has not been carefully estimated. Homozygous HCP is rare and presents during childhood. Harderoporphyria, a biochemically distinguishable variant of HCP, has been recognized in heteroallelic and homoallelic forms.

Pathology and Pathogenesis

Increased ALA and PBG during acute attacks of HCP may be explained by induction of ALAS1 and by the normally relatively low activity of PBGD in the liver. Hepatic ALAS1 is increased during acute attacks but is normal when the disease is latent and porphyrin precursor excretion is normal. Because coproporphyrinogen III concentration in the liver is probably less than the K_m for CPOX, the reaction rate is likely to be determined in part by substrate concentration. The substrate coproporphyrinogen appears to be lost more readily from the liver cell than, for example, uroporphyrinogen, especially when heme synthesis is stimulated. Coproporphyrin and coproporphyrinogen are both transported into bile and excreted in urine and do not appear to accumulate in the liver in HCP.

Clinical Manifestations

Symptoms are identical to those of AIP except that attacks are generally milder, and cutaneous lesions that resemble those in PCT develop occasionally. *Severe motor neuropathy and respiratory paralysis can occur*. HCP is almost always latent before puberty, and symptoms are most common in adult women. Attacks are precipitated by the same factors that cause attacks in AIP, including fasting, OCs, and hormone increases during the luteal phase of the menstrual cycle. Concomitant liver diseases may increase porphyrin retention and photosensitivity. The risk of hepatocellular carcinoma is increased.

The clinical features of homozygous HCP or harderoporphyria begin in early childhood and include jaundice, hemolytic anemia, hepatosplenomegaly, and skin photosensitivity. These symptoms are generally quite distinct from those seen in heterozygotes. Hematologic features are particularly characteristic in harderoporphyria.

Laboratory Findings

The porphyrin precursors ALA and PBG are increased during acute attacks in HCP but may decrease more rapidly than in AIP. Marked increases in coproporphyrin III in urine and feces are more persistent in HCP. Plasma porphyrins are usually normal or only slightly increased.

In homozygous cases, porphyrin excretion may be more increased and is accompanied by substantial increases in erythrocyte zinc protoporphyrin. Harderoporphyria is characterized by a marked increase in fecal excretion of harderoporphyrin (tricarboxyl porphyrin) and in coproporphyrin.

Diagnosis and Differential Diagnosis

The diagnosis of HCP is readily established in patients with clinically manifest disease, although urinary ALA, PBG, and uroporphyrin may revert to normal more quickly than in AIP. Urinary coproporphyrin III is increased. Urinary porphyrins, especially coproporphyrin, can be increased in many medical conditions (e.g., liver disease), and small increases that are not diagnostically significant may lead to an incorrect diagnosis of HCP. Fecal porphyrins are mostly coproporphyrin (isomer III) in HCP, whereas in VP, coproporphyrin III and protoporphyrin are often increased approximately equally. Plasma porphyrins are usually normal in HCP and increased in VP.

The ratio of fecal coproporphyrin III to coproporphyrin I is especially sensitive for detecting latent heterozygotes (especially in adults). Assays for CPOX, a mitochondrial enzyme, require cells such as lymphocytes and are not widely available. Identification of a *CPOX* pathogenic variant in an index case greatly facilitates screening family members.

Treatment and Prognosis

Acute attacks of HCP are treated as in AIP, which includes IV hemin and identifying and avoiding precipitating factors. Phlebotomy and chloroquine are not effective. GnRH analogs can be effective for prevention of cyclic attacks. The prognosis is generally better than in AIP. Givosiran, an siRNA therapeutic agent, has been approved for the prevention of acute attacks in all acute hepatic porphyrias, although experience in HCP is limited.

Prevention and genetic counseling are the same as in other acute porphyrias.

VARIEGATE PORPHYRIA

This hepatic porphyria is caused by a deficiency of protoporphyrinogen oxidase (PPOX), which is inherited as an autosomal dominant trait. The disorder is termed *variegate* because it can present with neurologic or cutaneous manifestations or both. Other terms have included *porphyria variegata, protocoproporphyria,* and *South African genetic porphyria.* Rare cases of homozygous VP are symptomatic in childhood.

Etiology

PPOX is approximately half-normal in all cells studied in patients with VP. The enzyme is more markedly deficient in rare cases of homozygous VP, with approximately half-normal enzyme activity in parents.

Human PPOX is a homodimer that contains flavin adenine dinucleotide and is localized to the cytosolic side of the inner mitochondrial membrane. Membrane-binding domains may be docked onto human FECH, the next enzyme in the pathway, which is embedded in the opposite side of the membrane. PPOX catalyzes the oxidation of protoporphyrinogen IX to protoporphyrin IX by the removal of six hydrogen atoms. The enzyme requires molecular oxygen. The substrate is readily oxidized nonenzymatically to protoporphyrin under aerobic conditions or if exported into the cytosol. PPOX is highly specific for protoporphyrinogen IX and is inhibited by tetrapyrroles such as heme, biliverdin, and bilirubin and by certain herbicides that cause protoporphyrin to accumulate and induce phototoxicity in plants. Inhibition by bilirubin may account for decreased PPOX activity in Gilbert disease.

The human *PPOX* gene consists of one noncoding and 12 coding exons. Many *PPOX* pathogenic variants have been reported in VP families. A missense pathogenic variant, R59W, is prevalent in South Africa. No convincing genotype-phenotype correlations have been identified. Pathogenic variants in homozygous cases of VP are more likely to encode enzyme proteins with residual activity.

Epidemiology

VP is less common than AIP in most countries. The R59W pathogenic variant is highly prevalent in South African Whites (3 in 1,000 in this population). This example of "genetic drift" or founder effect has been traced to a man or his wife who emigrated from Holland to South Africa in 1688. In Finland, prevalence is 1.3 in 100,000 people and is about as common as AIP.

Pathology and Pathogenesis

Acute attacks develop in a minority of heterozygotes for PPOX deficiency and are often attributable to drugs, steroids, and nutritional factors that play a role in other acute porphyrias. Protoporphyrinogen IX accumulates and undergoes autoxidation to protoporphyrin IX. Coproporphyrinogen III accumulates, perhaps as the result of a close functional association between PPOX in the inner mitochondrial

membrane and CPOX in the intermembrane space. Liver porphyrin content is not increased. The increased porphyrin content in plasma consists of porphyrin-peptide conjugates, which may be formed from protoporphyrinogen. Increased ALA and PBG during acute attacks may be explained, as in HCP, by induction of ALAS1 by exacerbating factors and by the normally relatively low activity of PBGD in liver. Furthermore, PBGD is inhibited by protoporphyrinogen, the substrate for PPOX.

Clinical Manifestations

Symptoms develop in some heterozygotes after puberty. Neurovisceral symptoms occurring as acute attacks are identical to AIP but are generally milder and less often fatal. Drugs, steroids, and nutritional alterations such as fasting, which are harmful in AIP, can also induce attacks of VP. Attacks occur equally in males and females, at least in South Africa. Cutaneous fragility, vesicles, bullae, hyperpigmentation, and hypertrichosis of sun-exposed areas are much more common than in HCP. They are likely to occur apart from and to be longer lasting than the neurovisceral symptoms. OCs can precipitate cutaneous manifestations. Acute attacks have become less common, and skin manifestations are more frequently the initial presentation; this may result from earlier diagnosis and counseling. The risk of hepatocellular carcinoma is increased.

Symptoms of homozygous VP begin in infancy or childhood. These children generally have severe photosensitivity, neurologic symptoms, seizures, developmental disturbances, and sometimes growth retardation, but they do not have acute attacks.

Laboratory Findings

Urinary ALA, PBG, and uroporphyrin are increased during acute attacks, but often less so than in AIP, and may be normal or only slightly increased during remission. Plasma porphyrins, urinary coproporphyrin III, and fecal coproporphyrin III and protoporphyrin are more persistently increased between attacks. The pattern of urinary porphyrins can sometime resemble that seen in PCT. Erythrocyte zinc protoporphyrin levels are greatly increased in homozygous VP and may be modestly increased in heterozygous cases.

Diagnosis and Differential Diagnosis

VP is readily distinguished biochemically from AIP and HCP, which also present with acute attacks and increases in PBG. Plasma porphyrin analysis is especially useful because the plasma porphyrins in VP are tightly protein bound, resulting in a characteristic fluorescence emission spectrum at neutral pH. Fecal porphyrins are increased, with approximately equal amounts of coproporphyrin III and protoporphyrin. Fluorometric detection of plasma porphyrins is more sensitive than stool porphyrin analysis in asymptomatic VP. PPOX assays using cells that contain mitochondria, such as lymphocytes, are sensitive for identifying asymptomatic carriers but are not widely available. Knowing the *PPOX* pathogenic variant in an index case enables the identification of relatives who carry the same pathogenic variant.

Treatment

Acute attacks are treated as in AIP. Hemin is beneficial for acute attacks but not for cutaneous symptoms. Light protection is important in patients with skin manifestations, using long-sleeved clothing, gloves, a broad-brimmed hat, and opaque sunscreen preparations. Exposure to short-wavelength UV light, which does not excite porphyrins, may increase skin pigmentation and provide some protection. Phlebotomy and chloroquine are not effective. Surprisingly, oral activated charcoal was reported to increase porphyrin levels and worsen skin manifestations.

Prognosis and Prevention

The outlook of patients with VP has improved, which may be attributed to improved treatment, earlier diagnosis, and detection of latent cases. Cyclic acute attacks in women can be prevented with a GnRH analog, as in AIP. Givosiran, an siRNA therapeutic agent, has been approved for the prevention of acute attacks in all acute hepatic porphyrias, although experience in VP is limited. A diagnosis of VP or any other acute porphyria should not lead to difficulty obtaining insurance, because the prognosis is usually good once the diagnosis is established.

Genetic counseling is the same as in other acute porphyrias.

ERYTHROPOIETIC PROTOPORPHYRIA AND X-LINKED PROTOPORPHYRIA

These forms of protoporphyria are genetically distinct but have essentially the same phenotype. In EPP, an autosomal recessive disorder, protoporphyrin accumulates as the result of a marked deficiency of FECH, the last enzyme in the heme biosynthetic pathway, because of *FECH* pathogenic variants. EPP is sometimes termed *erythrohepatic protoporphyria,* although the liver does not contribute substantially to production of excess protoporphyrin. XLP is the most recently described porphyria, in which gain-of-function *ALAS2* pathogenic variants lead to overproduction of ALA in the marrow, where it is metabolized to excess amounts of protoporphyrin.

Etiology

Ferrochelatase (FECH), the enzyme that is deficient in EPP, catalyzes the final step in heme synthesis, which is insertion of ferrous iron (Fe^{2+}) into protoporphyrin IX (see Fig. 112.1). The enzyme is also termed *heme synthetase* or *protoheme ferrolyase.* The human enzyme is a dimer, and each homodimer contains a [2Fe-2S] cluster, which may have a role in bridging homodimers. FECH is found in the mitochondrial inner membrane, where its active site faces the mitochondrial matrix. It may be associated with complex I of the mitochondrial electron transport chain, and the ferrous iron substrate may be produced on nicotinamide adenine dinucleotide oxidation. FECH is specific for the reduced form of iron but can use other metals, such as Zn^{2+} and Co^{2+}, and other dicarboxyl porphyrins. Accumulation of metal-free protoporphyrin rather than zinc protoporphyrin in EPP indicates that formation of the latter is dependent on FECH activity in vivo.

The human *FECH* gene has a single promoter sequence and contains 11 exons. Two mRNAs of 1.6 and 2.5 kb were described, which may be explained by the use of two alternative polyadenylation signals. The larger transcript is more abundant in murine erythroid cells, suggesting erythroid-specific regulation of FECH. A variety of *FECH* pathogenic variants have been reported in EPP, including missense, nonsense, and splicing pathogenic variants; small and large deletions; and an insertion.

The inheritance of two alleles associated with reduced FECH activity is required for disease expression. This is consistent with FECH activities as low as 15–25% of normal in EPP patients. In most patients, a pathogenic variant on one *FECH* allele is combined with a common variant affecting the other allele. This common-variant *FECH* allele (IVS3-48T>C) produces less-than-normal amounts of enzyme because it expresses an aberrantly spliced mRNA that is degraded by a nonsense-mediated RNA decay mechanism. The IVS3-48T>C *FECH* variant by itself does not cause disease, even when homozygous. In a few families, two severe *FECH* pathogenic variants have been found without the IVS3-48T>C allele. EPP with autosomal recessive inheritance occurs naturally in cattle and in mouse models.

XLP is associated with gain-of-function deletions in the last exon of *ALAS2*. These lesions delete the last 10-20 amino acids of the ALAS2 polypeptide and apparently make the enzyme more stable. Metal-free protoporphyrin predominates in erythrocytes in these cases, but because FECH activity is normal, the proportion of zinc protoporphyrin is greater than in EPP. XLP accounts for approximately 2% of cases with the EPP phenotype in Europe and approximately 10% of cases in North America.

EPP is sometimes associated with myelodysplastic or myeloproliferative disorders and expansion of a clone of hematopoietic cells with deletion of one *FECH* allele or with other *FECH* pathogenic variants. In such cases there is late onset of the disease.

Epidemiology

EPP is the most common porphyria to cause symptoms in children but is often not diagnosed until adult life. Overall, it is the third most

common porphyria, although its prevalence is not precisely known (see Table 112.2). An analysis from the UK biobank exome sequencing data suggests that EPP is 1.7-3.0 times more common than previously thought in the UK. It is described mostly in Whites but occurs in other ancestries. The IVS3-48T>C splice variant is common in Whites and Japanese but rare in Africans, which explains lower disease prevalence in populations of African origin.

Pathology and Pathogenesis

FECH is deficient in all tissues in EPP, but bone marrow reticulocytes are thought to be the primary source of the excess protoporphyrin, some of which enters plasma and circulates to the skin. Circulating erythrocytes are no longer synthesizing heme and hemoglobin, but they contain excess free protoporphyrin, which also contributes. In XLP caused by terminal deletions in exon 11 of *ALAS2,* all intermediates of the heme pathway are overproduced and ultimately accumulate in bone marrow erythroblasts as protoporphyrin. FECH is not deficient in XLP, so this enzyme chelates some of the excess protoporphyrin with zinc. An aberrantly spliced mitoferrin transcript, which limits iron transport into mitochondria, has also been described in EPP. The liver functions as an excretory organ rather than a major source for excess protoporphyrin. FECH deficiency in the skin and liver may be important, however, because tissue transplantation studies in mice suggest that skin photosensitivity and liver damage occur only when FECH is deficient in these tissues.

Patients with EPP and XLP are maximally sensitive to light in the 400-nm range, which corresponds to the so-called *Soret band,* the narrow peak absorption maximum that is characteristic for protoporphyrin and other porphyrins. Having absorbed light, porphyrins enter an excited energy state and release energy as fluorescence, singlet oxygen, and other ROS. Resulting tissue damage is accompanied by lipid peroxidation, oxidation of amino acids, cross linking of proteins in cell membranes, and damage to capillary endothelial cells. Such damage may be mediated by photoactivation of the complement system and release of histamine, kinins, and chemotactic factors. Repeated acute damage leads to thickening of the vessel walls and perivascular deposits from accumulation of serum components. Deposition of amorphous material containing immunoglobulin, complement components, glycoproteins, acid glycosaminoglycans, and lipids occurs around blood vessels in the upper dermis.

There is little evidence for impaired erythropoiesis or hemolysis in EPP. However, mild anemia with microcytosis, hypochromia, and reticulocytosis is common. Iron accumulation in erythroblasts and ring sideroblasts has been noted in bone marrow in some patients. Decreased transferrin saturation and low or low-normal serum ferritin suggest iron deficiency. Iron status should be carefully evaluated in EPP patients. Poor response to oral iron supplements is described in EPP and is unexplained, because iron absorption is not impaired. Some patients report increased photosensitivity when given iron supplements, but whether this is from transient increases in porphyrins when iron deficiency is corrected and erythropoiesis increases is not known. Case reports suggest that iron supplementation decreases protoporphyrin and improves anemia, especially in patients with XLP. However, recent evidence suggests that iron deficiency may reduce protoporphyrin levels in some EPP patients.

Liver damage develops in a small proportion of EPP and XLP patients and is attributed to excess protoporphyrin, which is insoluble in water and excreted only by hepatic uptake and biliary excretion. Some may be reabsorbed by the intestine and undergo enterohepatic circulation. At very high levels, protoporphyrin is cholestatic, can damage cholangiocytes, and can accumulate in hepatocytes to form crystalline structures and impair mitochondrial function.

Clinical Manifestations

Symptoms of cutaneous photosensitivity begin in childhood and consist of acute pain and itching, often occurring within minutes of sunlight exposure and followed by redness and swelling with continued exposure (Fig. 112.7). Petechiae and purpuric lesions may be seen, but blisters are rare. Swelling may resemble angioneurotic edema and *solar urticaria.* Symptoms are usually worse in the spring and summer. Chronic changes may include lichenification, leathery pseudovesicles, labial grooving, and nail changes, but changes in pigmentation and pronounced scarring are unusual. Although physical findings in EPP and XLP may not be impressive, efforts to avoid sunlight and resulting symptoms significantly impair quality of life. An association between EPP caused by pathogenic variants affecting both FECH alleles and seasonal palmar keratoderma is unexplained. Neuropathy develops only in some patients with severe hepatic decompensation. XLP males have a more severe phenotype with higher protoporphyrin levels than most EPP patients. XLP females have a variable clinical presentation—some with no symptoms or mild symptoms and others with severe symptoms similar to XLP males. This variability in females is likely the result of random X chromosome inactivation.

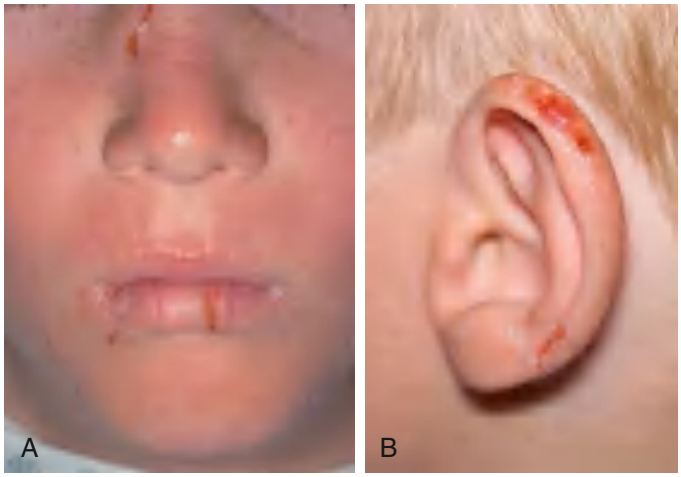

Fig. 112.7 Erythropoietic protoporphyria (EPP). **A,** Linear erosions of the lateral nasal bridge and lower lip in a patient with EPP. **B,** Erosions with crusting on the left helix of a patient with EPP. (*From Horner ME, Alikhan A, Tintle S, et al. Cutaneous porphyrias. Part 1. epidemiology, pathogenesis, presentation, diagnosis, and histopathology. Int J Dermatol. 2013;52:1464–1480, Figs. 7 and 8.*)

Unless hepatic or other complications develop, protoporphyrin levels and symptoms of photosensitivity remain remarkably stable for many years in most patients. Factors that exacerbate hepatic porphyrias play little or no role in EPP or XLP. Erythrocyte protoporphyrin levels may decrease and sunlight tolerance may improve during pregnancy, which is unexplained.

Laboratory Findings

Protoporphyrin is substantially increased in circulating erythrocytes in EPP and consists almost entirely of metal-free protoporphyrin. In XLP, both zinc protoporphyrin and metal-free protoporphyrin are increased, although the latter still predominates. Protoporphyrin is also increased in bone marrow, plasma, bile, and feces. Other porphyrins and porphyrin precursors are normal in uncomplicated EPP and XLP.

Diagnosis and Differential Diagnosis

A diagnosis of EPP is confirmed biochemically by finding a substantially elevated concentration of total erythrocyte protoporphyrin, which is predominantly (at least 85%) metal-free and not complexed with zinc. In XLP, both metal-free and zinc-complexed protoporphyrins are elevated. Erythrocyte total protoporphyrin levels are, on average, higher in XLP and more variable between individuals in EPP, possibly reflecting differences in severity of the many reported *FECH* pathogenic variants. Erythrocyte zinc protoporphyrin concentration is increased with little increase in metal-free protoporphyrin in homozygous porphyrias (except CEP), iron deficiency, lead poisoning, anemia of chronic disease, hemolytic conditions, and many other erythrocytic disorders. Measurement of FECH activity requires cells containing mitochondria and is not widely available.

Plasma total porphyrin concentration is often less increased in EPP than in other cutaneous porphyrias and may be normal. Great care must be taken to avoid light exposure during sample processing, because plasma porphyrins in EPP are particularly subject to photodegradation. Urinary porphyrin precursors and porphyrins are not increased.

DNA studies are strongly recommended for confirming *FECH* or *ALAS2* pathogenic variants and for genetic counseling.

Life-threatening **protoporphyric hepatopathy** is characterized by greater increases in erythrocyte and plasma protoporphyrin levels, increased photosensitivity, and either chronically abnormal liver function tests or rapidly progressive hepatic failure. Presumably this is heralded by increases above the patient's baseline erythrocyte and plasma porphyrin levels, but this has not been documented, because most such patients have not had sufficiently long-term determinations of porphyrin values. Increases in urinary porphyrins, especially coproporphyrin, in this setting are attributable to liver dysfunction.

Complications

There is an increased risk of biliary stones, which contain protoporphyrin and are sometimes symptomatic, requiring cholecystectomy. Protoporphyric hepatopathy occurs in <5% of protoporphyria patients, including children, and may be chronic or progress rapidly to death from liver failure. Rarely, hepatopathy is the major presenting feature of EPP or XLP. Protoporphyric hepatopathy can cause acute upper abdominal pain suggesting biliary obstruction, and unnecessary laparotomy to exclude this possibility can be detrimental. Other types of liver disease, such as viral hepatitis or alcohol- or drug-induced liver disease, must be excluded, or may contribute to the development of protoporphyric hepatopathy. Whether iron deficiency may contribute is unclear. Liver histology shows marked deposition of protoporphyrin as inclusions in liver cells and bile canaliculi. The bone marrow is probably the major source of protoporphyrin, even in EPP patients with hepatic failure.

Treatment

Exposure to sunlight should be avoided, which is aided by wearing closely woven clothing. Beta-carotene, oral cysteine, and vitamin C have no proven efficacy. One report suggested that high doses of cimetidine were effective in reducing symptoms in three children with EPP, but no objective clinical evidence of efficacy was presented.

Increasing skin melanin by narrow-band UV-B phototherapy may improve sunlight tolerance. Studies in the United States and Europe of afamelanotide, a synthetic analog of melanocyte-stimulating hormone, darkened the skin, increased pain-free sun exposure, and improved quality of life in patients with protoporphyria. This drug is approved for use in adults in Europe and the United States. Dersimelagon, an orally administered small molecule and a selective melanocortin-1 receptor (MC1R) agonist that increases skin melanin, is currently in phase 3 trials in EPP and XLP in the United States and other countries.

Drugs or hormone preparations that impair hepatic excretory function should be avoided. Iron deficiency should be corrected, particularly in XLP. Vitamin D supplementation and hepatitis A and B vaccination are recommended.

Treatment of protoporphyric hepatopathy must be individualized and exclude other causes of liver disease. Spontaneous resolution may occur, especially if another reversible cause of liver dysfunction, such as viral hepatitis or alcohol abuse, is contributing. In patients with severe hepatic decompensation, combined treatment with plasmapheresis, transfusion to correct anemia and suppress erythropoiesis, IV hemin to suppress erythroid and possibly hepatic protoporphyrin production, ursodeoxycholic acid, vitamin E, and cholestyramine may be beneficial and bridge patients to liver transplantation.

Motor neuropathy resembling that seen in acute porphyrias sometimes develops in protoporphyria patients with liver disease before or after transfusion or liver transplantation and is sometimes reversible. Artificial lights, such as operating room lights during liver transplantation or other surgery, may cause severe photosensitivity, with extensive burns of the skin and peritoneum and damage to circulating erythrocytes.

Although liver disease may recur in the transplanted liver as a result of continued bone marrow production of excess protoporphyrin, outcomes are comparable to transplantation for other types of liver disease. Bone marrow transplantation can be considered after liver transplantation if a suitable donor is available.

Prognosis

Typical EPP patients have lifelong photosensitivity but can otherwise expect normal longevity. Protoporphyric liver disease is often life-threatening; however, the incidence is low.

Prevention and Genetic Counseling

Symptoms can be prevented by avoiding sunlight. Avoiding agents that may cause liver damage may help prevent liver complications. Opinions vary on the value of iron replacement, and this is currently under study.

DNA studies to identify *FECH* pathogenic variants, the common IVS3–48T>C *FECH* hypoexpression allele, or *ALAS2* exon 11 deletions are important for genetic counseling. When EPP is caused by a severe *FECH* pathogenic variant and the common IVS3–48T>C *FECH* allele, DNA studies in the spouse to determine the presence, or more likely the absence, of the hypoexpression allele can predict whether offspring are at risk for EPP. EPP may improve during pregnancy.

DUAL PORPHYRIA

An unusual pattern of porphyrin precursors and porphyrins has led to documentation of pathogenic variants of two heme pathway enzymes. One such patient presented with acute porphyria and had heterozygous pathogenic variants of both *CPOX* and *ALAD*. Another had symptoms of AIP and PCT and was reported to have both *HMBS* and *UROD* pathogenic variants. In other reported cases, one or both enzyme deficiencies were based on enzyme measurements.

PORPHYRIA RESULTING FROM TUMORS

Erythropoietic porphyrias can develop late in life in patients with myelodysplastic or myeloproliferative diseases and clonal expansion of erythroid cells with an inherited or somatic pathogenic variant of an enzyme in the heme biosynthetic pathway.

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Chapter 113

Hypoglycemia

Katherine Lord and Diva D. De León-Crutchlow

Glucose plays a central role in fuel metabolism and energy storage in the body. It provides 38 mol of adenosine triphosphate (ATP) per mole of glucose oxidized. Glucose is also essential for energy metabolism in the brain, where it is the preferred substrate. Its use accounts for nearly all the brain's oxygen consumption. Cerebral transport of glucose is a glucose transporter-1 (GLUT-1)–facilitated diffusion process that is dependent on blood glucose concentration and not regulated by insulin. *Low concentrations of blood glucose result in cerebral glucopenia, energy failure, and brain injury.* An elaborate regulatory system has evolved to maintain glucose homeostasis and prevent plasma glucose from falling precipitously to levels that impair brain function. The defense against hypoglycemia includes the autonomic nervous system and hormones that act in concert to enhance glucose production through glycogenolysis and gluconeogenesis, while simultaneously limiting peripheral glucose use, which conserves glucose for cerebral metabolism. With prolonged fasting, fat stores are mobilized via lipolysis, and fatty acid oxidation in the liver results in the generation of ketone bodies, an alternative fuel source for the brain (Fig. 113.1). Hypoglycemia results from a failure in one or several of these fasting mechanisms that normally integrate glucose homeostasis.

DEFINITION

Hypoglycemia is defined as a plasma glucose concentration low enough to cause signs and symptoms of brain dysfunction (Fig. 113.2). However, a numerical value can be difficult to define because the brain responses to hypoglycemia occur across a range of plasma glucose concentrations. In young children, signs and symptoms of hypoglycemia are suggestive but nonspecific; thus the reliance on signs and symptoms to indicate hypoglycemia in this age-group may be problematic (Table 113.1).

In older children, evidence of hypoglycemia is based on **Whipple's triad**: (1) signs and symptoms consistent with hypoglycemia, (2) a low plasma glucose concentration, and (3) resolution of the symptoms with normalization of the plasma glucose concentration.

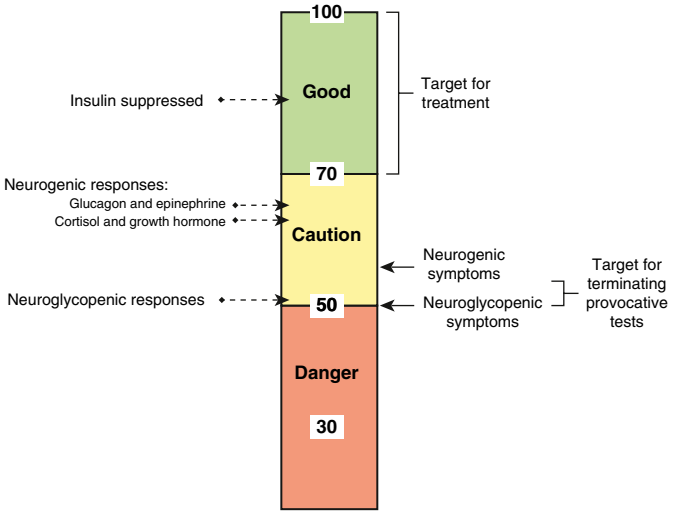

Fig. 113.2 Interpreting glucose levels and glucose treatment targets. Glucose thresholds are shown for suppression of insulin secretion, neurogenic (neuroendocrine hormone-mediated) responses, and neuroglycopenic (impaired cognition) responses. Colors show the normal range *(green)*, the range for symptoms caused by activation of sympathetic nervous system *(yellow)*, and the range for impaired neuronal function *(red)*. (*From Stanescu DL, Stanley CA. Advances in understanding the mechanism of transitional neonatal hypoglycemia and implications for management. Clin Perinatol. 2022;49:55–72, Fig. 2.*)

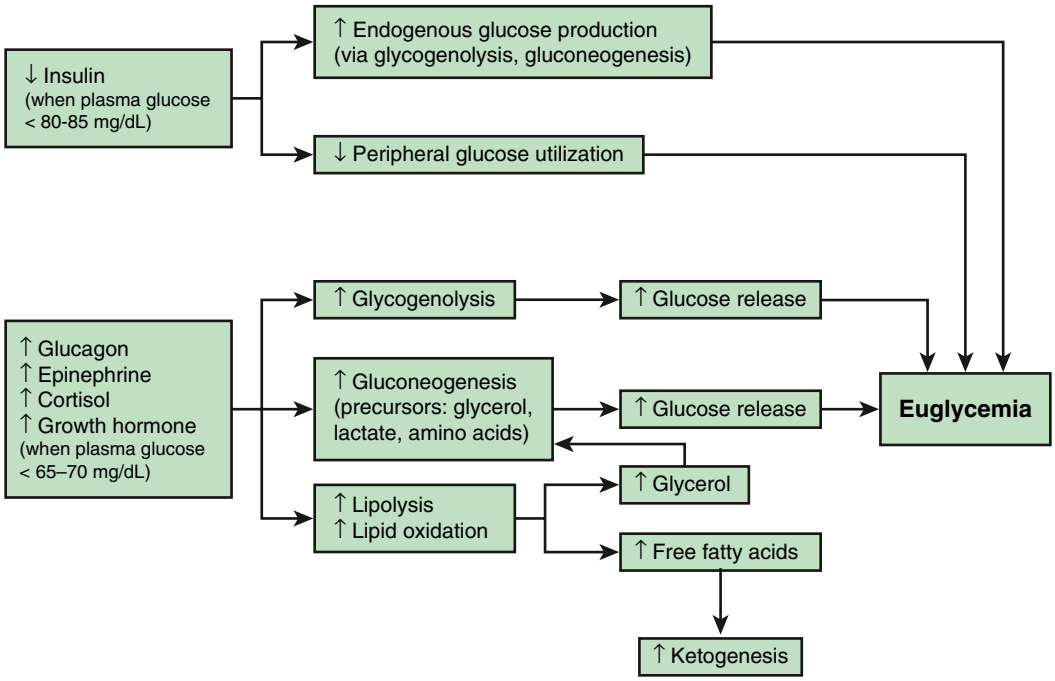

Fig. 113.1 The metabolic response to fasting. With fasting, declining glucose levels result in suppression of insulin and a rise of the counter-regulatory hormones. These changes in hormone levels lead to increased glucose output from the liver via glycogenolysis and gluconeogenesis. With prolonged fasting, counter-regulatory hormones stimulate lipolysis, which results in generation of glycerol (a key precursor in the gluconeogenic pathway) and, through ketogenesis, ketone bodies, a critical alternative fuel.

Table 113.1 Manifestations of Hypoglycemia in Childhood

FEATURES ASSOCIATED WITH ACTIVATION OF AUTONOMIC NERVOUS SYSTEM AND EPINEPHRINE RELEASE*
Anxiety
Perspiration
Palpitation (tachycardia)
Pallor
Tremulousness
Weakness
Hunger
Nausea
Emesis
FEATURES ASSOCIATED WITH CEREBRAL GLUCOPENIA
Headache
Mental confusion
Visual disturbances (↓ acuity, diplopia)
Inability to concentrate
Dysarthria
Staring
Paresthesias
Dizziness
Amnesia
Ataxia
Refusal to feed
Somnolence, lethargy
Seizures
Coma
Stroke, hemiplegia, aphasia
Decerebrate or decorticate posture

*Some of these features will be attenuated if the patient is receiving β-adrenergic blocking agents.

SIGNIFICANCE AND SEQUELAE

Brain metabolism uses the majority of endogenous hepatic glucose production in neonates, infants, and children. Because the brain grows most rapidly in the first year of life and a larger proportion of glucose turnover is used for brain metabolism, hypoglycemia in infants and children can affect brain development and function and can result in developmental delays and learning disabilities. The brain also has the capacity to absorb and oxidize ketone bodies. However, the capacity of the liver to produce ketone bodies is limited in the immediate newborn period. This is especially restricted in the presence of **hyperinsulinism (HI)**, which acutely inhibits hepatic glucose output, lipolysis, and ketogenesis, thereby depriving the brain of any alternative fuel sources. Furthermore, although the brain can metabolize ketones, these alternative fuels cannot completely replace glucose as an essential central nervous system (CNS) fuel. The deprivation of the brain's major energy source during hypoglycemic events has predictable adverse consequences on brain metabolism and growth. These arise from decreased brain oxygen consumption and increased breakdown of endogenous structural brain components, with impairment and loss of functional membrane integrity.

The major long-term sequelae of hypoglycemia are developmental delays, learning disabilities, epilepsy, and behavioral issues. A prospective cohort study of neonates at risk for hypoglycemia found that at 4 years of age, children who had experienced symptomatic hypoglycemia during the neonatal period had a two- to threefold increased risk of low executive and visual-motor function with greater impairment in children with severe or recurrent episodes. Cross-sectional studies in children with HI have found that 26–48% of children with both transient and persistent forms of HI have abnormal neurodevelopment. Children with HI have the highest risk of neurologic damage given the severity of the hypoglycemia in the condition and their inability to generate ketones as an alternative fuel for the brain. In addition, hypoglycemia associated with hypoxic-ischemic encephalopathy increases the risk of CNS sequalae. However, there are no precise data relating the duration or severity of hypoglycemia to subsequent neurologic development of children in a predictable manner. Nonetheless, early detection of hypoglycemia and effective treatment are critical to mitigating the risk of brain damage and developmental delays. Many neonates may have asymptomatic hypoglycemia identified by routine screening or laboratory testing. Although asymptomatic hypoglycemia in an otherwise healthy newborn is usually benign and transient, there remains a risk that if untreated (glucose gel, human milk, formula feedings), the hypoglycemia may become symptomatic. Hypoglycemia in older children may also produce long-term neurologic defects through neuronal death, mediated in part by cerebral excitotoxins released during hypoglycemia.

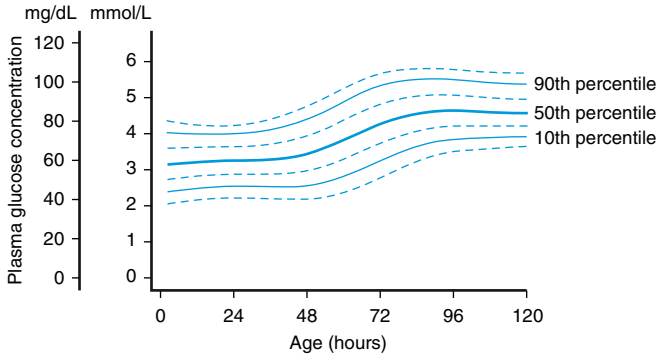

Fig. 113.3 Glucose percentiles in healthy term infants. (*Modified from Harris DL, Weston PJ, Gamble GD, Harding JE. Glucose profiles in healthy term infants in the first 5 days: the Glucose Well Babies (GLOW) study. J Pediatr. 2020;223:34–41, Fig. 2.*)

PHYSIOLOGIC MECHANISMS OF GLUCOSE HOMEOSTASIS

Fetal glucose is derived entirely from the mother through placental-facilitated diffusion; therefore fetal glucose concentration reflects, but is slightly lower than, maternal glucose levels. After birth, plasma glucose decreases, reaching a nadir at about 2 hours of life and remaining below the normal adult range of 70-100 mg/dL for the first 3-4 days of life; this is known as *transitional hypoglycemia*. A study of healthy, full-term infants found a mean plasma glucose concentration of 59 ± 11 mg/dL during the first 48 hours of life, which then increased to 83 ± 13 mg/dL during days 4-5 of life (Fig. 113.3). Transitional asymptomatic hypoglycemia is likely caused by a persistence of fetal pancreatic β-cell fuel metabolism, which allows for the secretion of insulin at lower glucose concentrations to support insulin-mediated fetal growth. Beyond this transitional period, plasma glucose concentration and mechanisms that regulate glucose homeostasis in neonates are the same as in older children and adults.

During fasting, mechanisms responsible for the maintenance of glucose homeostasis include glycogenolysis, gluconeogenesis, lipolysis, and fatty acid oxidation, as well as the hormones that regulate the activation of these processes (see Fig. 113.1). Plasma glucose concentrations are maintained by both metabolism of the nutrients consumed in the immediate postprandial period and then by glycogenolysis and gluconeogenesis starting 2-4 hours after the meal. The liver of a 10-kg child contains 20-25 g of glycogen, which is sufficient to meet normal glucose requirements of 4-6 mg/kg/minute during an overnight fast. Glucose production during this time is augmented by gluconeogenesis (see Fig. 113.1). Defects in glycogenolysis or gluconeogenesis may not be manifested in infants until the frequent feeding pattern at 3- to 4-hour intervals ceases and infants sleep through the night, a situation usually present by 3-6 months of age.

With prolonged fasting, the glycogen stores are depleted, and energy metabolism is more dependent on fatty acid and ketone oxidation. **Lipolysis** generates fatty acids, which undergo β-oxidation in the liver to generate ketone bodies. Peripheral tissues use free fatty acids (FFAs) and ketones, whereas the CNS can use ketones for a

portion of its energy needs. Defects in β-oxidation and ketogenesis may not manifest until fasting exceeds the overnight period or, with illnesses, result in catabolism, fasting, and increased body energy requirements.

Amino acid precursors for gluconeogenesis are derived primarily from muscle protein. The muscle bulk of infants and small children is substantially smaller relative to body mass than that of adults, whereas glucose requirements per unit of body mass are greater in children. Therefore the ability to compensate for glucose deprivation by gluconeogenesis is more limited in infants and young children, as is the ability to withstand fasting for prolonged periods. The ability of muscle to generate alanine, the principal gluconeogenic amino acid, may also be limited.

The switch from glycogen synthesis during and immediately after meals to glycogen breakdown and later gluconeogenesis is governed by hormones, with insulin of central importance (see Fig. 113.1). After a meal, plasma insulin concentrations increase to peak levels of 5- to 10-fold greater than their normal baseline concentration, which serves to lower the plasma glucose through the enhancement of peripheral glucose uptake, activation of glycogen synthesis, and inhibition of gluconeogenesis. In addition, lipogenesis is stimulated, whereas lipolysis and ketogenesis are curtailed. During fasting, plasma insulin concentrations fall, and together with the rise of counter-regulatory hormones, result in maintenance of plasma glucose concentration (see Fig. 113.1).

These **counter-regulatory hormones**—glucagon, growth hormone, cortisol, and epinephrine—act synergistically and in concert to increase plasma glucose concentrations by activating glycogenolytic enzymes (glucagon, epinephrine); inducing gluconeogenic enzymes (glucagon, cortisol); inhibiting glucose uptake by muscle (epinephrine, growth hormone, cortisol); mobilizing amino acids from muscle for gluconeogenesis (cortisol); activating lipolysis and thereby providing glycerol for gluconeogenesis and fatty acids for ketogenesis (epinephrine, cortisol, growth hormone, glucagon); and inhibiting insulin release and promoting growth hormone and glucagon secretion (epinephrine).

CLINICAL MANIFESTATIONS

Clinical features of hypoglycemia fall into two categories: (1) symptoms and signs associated with the activation of the autonomic nervous system and epinephrine release **(autonomic symptoms)** and (2) symptoms and signs caused by decreased cerebral glucose use **(neuroglycopenic symptoms)** (see Table 113.1). In newborns and infants, symptoms and signs of hypoglycemia may be subtler and include cyanosis, apnea, hypothermia, hypotonia, irritability, jitteriness, poor feeding, lethargy, and seizures. *It is important to note that neonates and infants are frequently asymptomatic when hypoglycemic.*

DIAGNOSIS

Infants and children with a suspected hypoglycemia disorder require a timely and comprehensive diagnostic workup to establish the underlying cause and to initiate specific treatment, prevent hypoglycemia, and minimize the risk of brain damage and neurologic dysfunction. The Pediatric Endocrine Society provides guidelines that outline which children and adolescents should be evaluated for a hypoglycemia disorder:

- Older children and adolescents who demonstrate Whipple's triad
- Infants and younger children (who cannot communicate symptoms) with plasma glucoses <60 mg/dL on laboratory-quality assays
  - Neonates at high risk of a persistent hypoglycemic disorder
  - Glucose screening is recommended for those born large for gestational age, a history of perinatal stress, premature or postmature delivery, infants of diabetic mothers, family history of hypoglycemia disorder, or a congenital syndrome associated with hypoglycemia.
  - A persistent hypoglycemia disorder should be ruled out in those with severe hypoglycemia (symptomatic or requiring intravenous [IV] dextrose), inability to maintain plasma glucose >50 mg/dL (up to 48 hours of life) or >60 mg/dL (after 48 hours of life), family history of a genetic form of hypoglycemia, and those with a congenital syndrome associated with hypoglycemia.

Table 113.2 Clinical Features of Hypoglycemia Disorders

BIRTH HISTORY	
Large for gestational age	Congenital HI, BWS
Small for gestational age	Perinatal stress HI
Maternal gestational diabetes	Infant of diabetic mother, dominant K_{ATP} -HI
FAMILY HISTORY	
Sudden infant death	Fatty acid oxidation disorder
Diabetes*	HNF4A-HI, HNF1A-HI, dominant K_{ATP} -HI
PHYSICAL EXAM	
Hepatomegaly	Glycogen storage disease
Congenital heart disease	Perinatal stress HI, Kabuki or Turner syndrome
Midline defect	Hypopituitarism
Macroglossia	BWS
Hyperpigmentation	Primary adrenal insufficiency
Short stature	Growth hormone deficiency, glycogen storage disease

*Specifically, non–type 1 diabetes diagnosed at a younger age in lean individuals.
HI, Hyperinsulinism; BWS, Beckwith-Wiedemann syndrome; K_{ATP}, ATP-sensitive potassium channel; HNF, hepatocyte nuclear factor.

The etiology of hypoglycemia varies based on the age of the child, associated clinical and laboratory features, and more detailed diagnostic testing, including gene panels or whole exome sequencing (Tables 113.2, 113.3, 113.4, and 113.5).

A thorough history and physical exam may provide clinical clues to the underlying diagnosis (see Table 113.2). Evaluation for hypoglycemia disorders requires obtaining blood and urine at the time of hypoglycemia, ideally when the plasma glucose is <50 mg/dL. This "**critical sample**" is used to measure hormones, which regulate glucose metabolism, and metabolic fuels (Table 113.6, Fig. 113.4). The critical sample can be obtained during a spontaneous episode of hypoglycemia or during a supervised diagnostic fast. Given the likelihood of hypoglycemia, a **diagnostic fast** should be performed in a hospital setting with close supervision and placement of an IV catheter to facilitate obtaining the critical sample. Point-of-care glucose (and β-hydroxybutyrate if available) should be followed closely, so the samples can be obtained as soon as the plasma glucose falls below 50 mg/dL. The evaluation of neonates should not occur during the first 48-72 hours of life to avoid the period of transitional hypoglycemia.

After the critical sample is obtained and before the child is allowed to eat, a **glucagon stimulation test** should be performed to assess for hyperinsulinism. While the child is still hypoglycemic, 1 mg of glucagon is administered, and glucose is measured by a point-of-care meter every 10 minutes for a total of 40 minutes. A greater than 30-point rise in plasma glucose concentration is consistent with insulin excess or hyperinsulinism. If children do not have an increase in their glucose during the first 20 minutes of the test, the test should be terminated and the child should be given juice or IV dextrose as rescue therapy.

The levels of alternative fuels at the time of hypoglycemia (β-hydroxybutyrate, FFAs, and lactate) allow for the classification of the hypoglycemia disorders into four distinct groups (see Fig. 113.4). The results from the critical sample then allow for narrowing of the differential and guide additional testing. Interpretation of the critical sample results has several important caveats. Low cortisol and growth hormone levels on the critical sample are not diagnostic of a hormone deficiency, and in most cases, the appropriate (specific) stimulation testing is needed if these levels are low. Additionally, a low or undetectable insulin level does not rule out hyperinsulinism. Insulin concentrations can be affected by hemolysis, and assays have variable sensitivity for detecting insulin. Other biomarkers of insulin effect, such as β-hydroxybutyrate (to evaluate ketogenesis), FFAs (to evaluate lipolysis), and the glycemic response to glucagon (to evaluate glycogenolysis), should be used to assess for hyperinsulinism.

Table 113.3 Classification of Hypoglycemia in Infants and Children

NEONATAL TRANSITIONAL (ADAPTIVE) HYPOGLYCEMIA
Associated with inadequate substrate or immature enzyme function in otherwise normal neonates
Prematurity
Small for gestational age
Normal newborn

Transient Neonatal Hyperinsulinism
Infant of diabetic mother
Small for gestational age
Discordant twin
Birth asphyxia
Infant of toxemic mother

NEONATAL, INFANTILE, OR CHILDHOOD PERSISTENT HYPOGLYCEMIA
Hyperinsulinism (see Tables 113.4 and 113.5)
Counter-Regulatory Hormone Deficiency
Panhypopituitarism
Isolated growth hormone deficiency
Adrenocorticotropic hormone deficiency
Addison disease (including congenital adrenal hypoplasia, adrenal leukodystrophy, triple A syndrome, ACTH receptor deficiency, and autoimmune disease complex)
Epinephrine deficiency

Glycogenolysis and Gluconeogenesis Disorders
Glucose-6-phosphatase deficiency (GSD Ia)
Glucose-6-phosphate translocase deficiency (GSD Ib)
Amylo-1,6-glucosidase (debranching enzyme) deficiency (GSD III)
Liver phosphorylase deficiency (GSD VI)
Phosphorylase kinase deficiency (GSD IX)
Glycogen synthetase deficiency (GSD 0)
Fructose-1,6-diphosphatase deficiency
Pyruvate carboxylase deficiency
Galactosemia
Hereditary fructose intolerance

Lipolysis Disorders
Fatty Acid Oxidation Disorders
Carnitine transporter deficiency (primary carnitine deficiency)
Carnitine palmitoyltransferase-1deficiency
Carnitine translocase deficiency
Carnitine palmitoyltransferase-2 deficiency
Secondary carnitine deficiencies
Very long-, long-, medium-, and short-chain acyl-CoA dehydrogenase deficiency

OTHER ETIOLOGIES
Substrate-Limited Causes
Ketotic hypoglycemia
Poisoning—drugs
Salicylates
Alcohol
Oral hypoglycemic agents
Insulin
Propranolol
Pentamidine
Quinine
Disopyramide
Ackee fruit (unripe)—hypoglycin
Litchi-associated toxin (toxic hypoglycemic syndrome)
Vacor (rat poison)
Trimethoprim-sulfamethoxazole (with renal failure)
L-Asparaginase and other antileukemic drugs

Liver Disease
Reye syndrome
Hepatitis
Cirrhosis
Hepatoma

AMINO ACID AND ORGANIC ACID DISORDERS
Maple syrup urine disease
Propionic acidemia
Methylmalonic acidemia
Tyrosinosis
Glutaric aciduria
3-Hydroxy-3-methylglutaric aciduria

SYSTEMIC DISORDERS
Sepsis
Carcinoma/sarcoma (secreting—insulin-like growth factor II)
Heart failure
Malnutrition
Malabsorption
Antiinsulin receptor antibodies
Antiinsulin antibodies
Neonatal hyperviscosity
Renal failure
Diarrhea
Burns
Shock
Chiari malformation
Postsurgical complication
Pseudohypoglycemia (leukocytosis, polycythemia)
Excessive insulin therapy of insulin-dependent diabetes mellitus
Factitious disorder
Nissen fundoplication (dumping syndrome)
Falciparum malaria

GSD, Glycogen storage disease; HI, hyperinsulinemia; KATP, regulated potassium channel.

Table 113.4 Endocrine and Metabolic Causes of Hyperinsulinemic Hypoglycemia

TRANSIENT
Infant of diabetic mother
Perinatal asphyxia
Rhesus hemolytic disease
Intrauterine growth restriction
HNF4A / HNF1A

CONGENITAL
ABCC8 / KCNJ11 / GCK / GDH / HADH / HNF4A / HNF1A / UCP2 / SLC16A1 / PMM2 / HK1 / PGM1 / FOXA2 / CACNA1D / EIF2S3

OTHERS
Postprandial hyperinsulinemic hypoglycemia
Insulinoma
Munchausen by proxy
Exercise-induced hyperinsulinemic hypoglycemia

Modified from Güemes M, Rahman SA, Kapoor RR, et al. Hyperinsulinemic hypoglycemia in children and adolescents: recent advances in understanding of pathophysiology and management. *Rev Endo Metab Dis.* 2020;21:577–597, Table 1.

MANAGEMENT

Acute Treatment

A child presenting with hypoglycemia should be rapidly treated with oral carbohydrates or IV dextrose to normalize their plasma glucose (>70 mg/dL). If the child is asymptomatic or has mild symptoms and is capable of oral intake, the hypoglycemia can be treated with 15 grams of rapid-acting carbohydrates, such as 4 ounces of juice or two graham crackers. If a child is symptomatic or unable to tolerate oral intake (risk of aspiration, impending depressed level of consciousness), 2 mL/kg of dextrose 10% water (D10W) should be administered. After administration of the bolus, the child should be placed on an IV dextrose infusion (for infants, a glucose infusion rate of 5-6 mg/kg/minute and for older children 2-3 mg/kg/minute) to prevent recurrent hypoglycemia. Plasma glucose via point-of-care testing should be monitored every 15-20 minutes until it is >70 mg/dL, and then checks may be spaced once stable levels are demonstrated.

In an asymptomatic child with a plasma glucose <50 mg/dL, a critical sample can be obtained if the appropriate supplies and tubes are readily available. Otherwise, treatment of the hypoglycemia should not be delayed.

Table 113.5 Syndromic Forms of Hyperinsulinemic Hypoglycemia*

SYNDROME NAME	GENETIC ETIOLOGY *GENE* (LOCATION)	CLINICAL CHARACTERISTICS
PRENATAL AND POSTNATAL OVERGROWTH (MACROSOMIA)		
Beckwith-Wiedemann	(11p15)	Macroglossia, abdominal wall defects, ear lobe pits/creases, hemihypertrophy, tumor risk; IUGR if associated with placental mesenchymal dysplasia
Sotos	*NSD1* (5q35)	Macrocephaly, frontal bossing, pointed chin, developmental delay, tumor risk
Simpson-Golabi-Behmel	GPC3 (Xq26), GPC4 (Xp22)	Coarse facial features, broad feet, polydactyly, cryptorchidism, hepatomegaly, tumor risk
Perlman	*DIS3L2* (2q37)	Inverted V-shaped upper lip, prominent forehead, developmental delay, hypotonia, tumor risk
POSTNATAL GROWTH FAILURE (SHORT STATURE)		
Kabuki	*KMT2D* (12q13), *KDM6A* (Xp11.3)	Arched eyebrows, long eyelashes, developmental delay, fetal finger pads, scoliosis, heart defects, hypotonia
Costello	*HRAS* (11p15)	Deep palmar/plantar creases, developmental delay, coarse facial features, heart abnormalities, papillomas, tumor risk
CHROMOSOMAL ABNORMALITY		
Mosaic Turner	Loss of X in some cells	Milder Turner syndrome phenotype (short stature, coarctation of aorta, gonadal dysgenesis)
Patau	Trisomy 13	Developmental delay, microphthalmia, heart and neural defects
CONGENITAL DISORDERS OF GLYCOSYLATION		
Types 1a, 1b, and 1d	*PMM2* (16p13.2), *MPI* (15q24.1), *ALG3* (3q27.1)	Developmental delay, hypotonia, growth failure
CONTIGUOUS GENE DELETION AFFECTING THE *ABCC8* GENE		
Usher	11 genes	Hearing loss, visual impairment
ABNORMALITIES IN CALCIUM HOMOEOSTASIS		
Timothy	*CACNA1C* (12p13.33)	Long QT syndrome, syndactyly, developmental delay, immune deficiency
INSULIN RECEPTOR PATHOGENIC VARIANT		
Insulin resistance syndrome (leprechaunism)	*INS* (19p13)	Hypoglycemia and hyperglycemia, prenatal and postnatal growth restriction, elfin-like features, hirsutism
OTHER SYNDROMES		
Congenital central hypoventilation syndrome	*PHOX2B* (4p13)	Central hypoventilation, "box-shaped" face, neurocristopathies (Hirschsprung disease, tumor risk)

IUGR, intrauterine growth restriction.
*Various developmental syndromes have been described with the gene/s linked to the condition and the common clinical features.
Modified from Güemes M, Rahman SA, Kapoor RR, et al. Hyperinsulinemic hypoglycemia in children and adolescents: recent advances in understanding of pathophysiology and management. *Rev Endo Metab Dis.* 2020;21:577–597, Table 2.

Ongoing Management

Treatment goals of hypoglycemia disorders include maintaining euglycemia (plasma glucose >70 mg/dL), promoting normal development, and monitoring for medication side effects. Once the diagnostic evaluation identifies a specific hypoglycemia disorder, tailored treatment should be initiated promptly. Effective treatment allows the child to maintain euglycemia, both while eating an age-appropriate diet and fasting overnight. Continuous or forced feeds should not be used as treatment for hypoglycemia, as they result in long-term oral aversion and excessive weight gain. Additionally, hypoglycemia should not routinely be treated with steroids or cornstarch unless indicated as a treatment for specific disorders, such as adrenal insufficiency or glycogen storage disease. Home glucose monitoring is required for all children with hypoglycemia disorders and is particularly important during times of illnesses, as this may provoke additional hypoglycemia.

Management of individual hypoglycemia disorders is given in more detail in the following sections and in Table 113.7.

DISORDERS OF HYPOGLYCEMIA

Disorders of hypoglycemia can be classified by the metabolic fuel response to fasting (see Fig. 113.4).

Insulin-Mediated Disorders

Insulin-mediated disorders (see Table 113.4) are characterized by low plasma β-hydroxybutyrate and FFAs and a positive glycemic response to glucagon (Table 113.8).

Hyperinsulinism

HI is the most common cause of *persistent hypoglycemia* in infants and children. HI is caused by dysregulated insulin secretion by the pancreatic β-cells, resulting in severe and recurrent hypoglycemia (Fig. 113.5). HI can be categorized into three main forms: (1) perinatal stress-induced, (2) congenital or monogenic (see Table 113.4), and (3) syndromic (see Table 113.5).

Perinatal stress-induced HI (PSHI), the most common form of HI, occurs in the setting of stress on the fetus in utero or during delivery.

Table 113.6 Hypoglycemia Diagnostic Evaluation

"CRITICAL SAMPLE" TO BE OBTAINED WHEN PLASMA GLUCOSE <50 MG/DL	
LABORATORY TEST	INTERPRETATION
Comprehensive metabolic panel	Low HCO_3 suggests elevation of ketones or lactate Elevated liver function tests may indicate GSD
β-hydroxybutyrate	Low levels suggest insulin excess (most commonly HI) or FAO disorder Elevated levels suggest GSD, hormone deficiency, or ketone utilization disorder
Insulin C-peptide	Detectable levels consistent with insulin excess Detectable insulin with undetectable c-peptide is consistent with exogenous insulin
Cortisol Growth hormone	Low levels concerning for hormone deficiency; need stimulation testing to confirm
Lactate	Elevated levels concerning for disorder of gluconeogenesis
Ammonia	Elevation can be seen in forms of HI and in IEM
Acylcarnitine profile Free and total carnitine Urine organic acids	Abnormalities suggestive of FAO disorder
IGF-BP1	Low levels suggest insulin excess
GLUCAGON STIMULATION TEST	
Administer 1 mg of glucagon IV or IM when plasma glucose <50 mg/dL Check point-of-care glucose every 10 min for 40 min If glucose does not increase 20 points in 20 min, terminate test and feed child Interpretation: Increase in glucose by 30 points is consistent with insulin excess	

HCO_3, Bicarbonate; GSD, glycogen storage disorder; HI, hyperinsulinism; FAO, fatty acid oxidation; IEM, inborn errors of metabolism.

In a study of 514 neonates at risk of hypoglycemia, 19% of late-preterm and small-for-gestational-age (SGA) infants had recurrent hypoglycemia, which is concerning for PSHI. Common causes of PSHI include intrauterine growth restriction, being born SGA, maternal preeclampsia, birth asphyxia, and congenital heart disease. *Most infants with PSHI respond to diazoxide, although a subset, particularly those with liver dysfunction and hypoxic ischemic encephalopathy, may fail to respond.* PSHI typically resolves within the first 3-6 months of life.

Congenital HI is the result of genetic defects in the insulin secretory pathways of the β-cell. It is estimated to occur in 1:28,000-50,000 live births but may be as high as 1:3,000 in populations with a high frequency of consanguinity. The most common and severe type of congenital HI is caused by inactivating pathogenic variants of *ABCC8* and *KCNJ11,* which encode the ATP-sensitive potassium (K_{ATP}) channel of the β-cell. Infants born with K_{ATP}-HI are commonly large for gestation age and have high glucose requirements (glucose infusion rate [GIR] >10 mg/kg/min) (see Fig. 113.5). However, the spectrum of presentation is wide, and some infants have normal birthweights and lower glucose requirements. In addition to fasting hypoglycemia, children with this type of HI have protein-induced hypoglycemia, in which isolated protein ingestion leads to increased insulin secretion and low plasma glucose. K_{ATP}-HI is most frequently unresponsive to treatment with diazoxide, the only approved drug for HI, which acts on the K_{ATP} channel.

K_{ATP}-HI has two distinct histologic forms: a **diffuse form**, in which β-cells throughout the pancreas show evidence of hyperactivity, and a focal form, characterized by a localized area of β-cell overgrowth or adenomatosis. The diffuse form is caused by biallelic recessive pathogenic variants in *ABCC8* or *KCNJ11* or, less commonly, monoallelic dominant pathogenic variants of these genes. **Focal HI** occurs as a result of a "two-hit" mechanism: a paternally inherited recessive pathogenic variant in *ABCC8* or *KCNJ11* combined with somatic loss of the maternal 11p15 region, resulting in paternal uniparental isodisomy. Infants with the focal form are cured with resection of the focal lesion. In contrast, those with the diffuse form may require a palliative near-total pancreatectomy if intensive medical therapy fails to control the hypoglycemia (Fig. 113.6). Given the different treatment approaches and outcomes for the diffuse and focal forms of K_{ATP}-HI, distinguishing between the two is critical and is best done through genetic testing. A paternally inherited recessive pathogenic variant in *ABCC8* or *KCNJ11* has a 94% positive predictive value for focal K_{ATP}-HI.

In children with genetic testing consistent with the focal form, an 18-fluoro L-3,4-dihydroxyphenylalanine positron emission tomography (^{18}F-DOPA PET) scan is used before surgery to localize the focal lesion (see Fig. 113.6). Frozen section evaluation of biopsies taken during surgery is used to confirm the presence of the focal lesion and guides the extent of the resection. Children with the focal form of HI should receive care at specialized centers with ^{18}F-DOPA PET access and a multidisciplinary team of endocrinologists, surgeons, pathologists, and radiologists with expertise in HI. Children with the diffuse form also benefit from care at these centers because their management and the decision to pursue intensive medical therapy or a near-total pancreatectomy are complex.

The second most common form of congenital HI is caused by **dominant activating pathogenic variants of *GLUD1***, which encodes **glutamate dehydrogenase** (GDH). This protein regulates the first step of amino acid–stimulated insulin secretion in the β-cell. Children with GDH-HI or hyperinsulinism-hyperammonemia syndrome (HI/HA) have fasting hypoglycemia, significant protein-induced hypoglycemia, and elevated plasma ammonia concentration. Seizures, attention-deficit disorder, and learning disabilities also occur in this form of HI, and these neurologic issues do not seem to correlate with the degree of hypoglycemia or ammonia elevation. *This form of HI responds well to diazoxide.*

Activating pathogenic variants of *GCK*, which encodes **glucokinase**, cause an autosomal dominant form of congenital HI. Glucokinase is the key enzyme triggering glucose-mediated insulin secretion. Children with GCK-HI have fasting hypoglycemia of variable severity and diazoxide responsiveness. Severe cases may require a near-total pancreatectomy.

Pathogenic variants in *HNF4A* and *HNF1A,* which encode the transcription factors hepatic nuclear factors 4-alpha and 1-alpha, cause a dominant form of diazoxide-responsive HI. These infants may be large for gestational age and have high GIR requirements at birth. The HI resolves within the first several years of life. However, some individuals experience progressive β-cell failure and progress to an early-onset diabetes, known as *maturity-onset diabetes of the young (MODY).*

Syndromes associated with HI are increasingly being recognized (see Table 113.5). Beckwith-Wiedemann syndrome (BWS), an imprinting disorder, is characterized by macroglossia, lateralized overgrowth, omphalocele, and a predisposition to embryonal tumors (Fig. 113.7). Hyperinsulinism occurs in approximately 50% of infants with BWS, although most cases are mild and resolve within the first several weeks of life. These cases are typically responsive to diazoxide. Children with BWS caused by paternal uniparental isodisomy of 11p can have severe and persistent HI, which does not respond to diazoxide and may

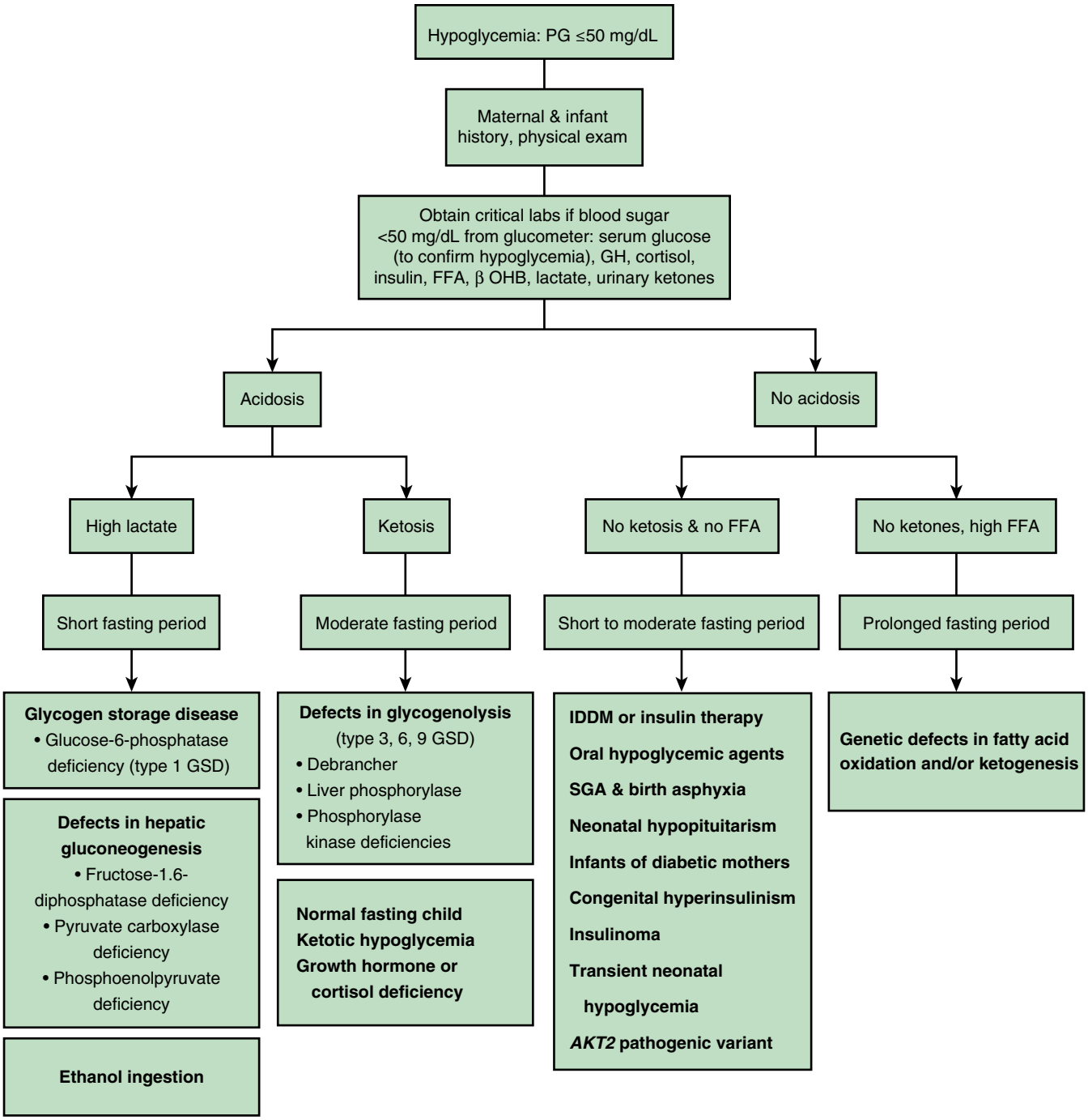

Fig. 113.4 Diagnostic algorithm for determining the etiology of hypoglycemia in children. OHB, Hydroxybutyrate; FFA, free fatty acid; GH, growth hormone, GSD, glycogen storage disease; PG, plasma glucose; IDDM, insulin-dependent diabetes mellitus. (*Modified from Melmed S, Auchus RJ, Goldfine AB, Koenig RJ, Rosen CJ. Williams Textbook of Endocrinology. 14th ed. Elsevier; 2020:1547, Fig. 38.14.*)

require a pancreatectomy. Other syndromes known to be associated with HI include Kabuki, Turner, and Rubinstein-Taybi. HI in these syndromes presents with variable degrees of severity and responsiveness to diazoxide.

After establishing a diagnosis of HI, a trial of diazoxide, the first-line agent for the treatment of HI, should be undertaken (Fig. 113.8). Diazoxide opens the K_{ATP} channel, inhibiting insulin secretion. Children are considered responsive to diazoxide if the cardinal feature of HI, hypoketotic hypoglycemia, is corrected. This is assessed by demonstrating that the child can maintain plasma glucose >70 mg/dL both while feeding and during an overnight fast and/or plasma β-hydroxybutyrate concentration increases to >2 mmol/L before plasma glucose decreases below 50-60 mg/dL during fasting. *Failure to respond to diazoxide strongly suggests K_{ATP}-HI, and those children require expedited genetic testing for ABCC8 and KCNJ11 to determine their risk for focal HI.* Thus the assessment of the response to diazoxide has important therapeutic and diagnostic implications.

Somatostatin analogs are used off-label as second-line agents for the treatment of diazoxide-unresponsive diffuse HI. Octreotide requires multiple daily injections, and its effectiveness is limited by

Table 113.7 Treatments for Hypoglycemia Disorders

DRUG/THERAPY	INDICATION	DOSE (ROUTE OF ADMINISTRATION)	SIDE EFFECTS/COMMENTS
Diazoxide	HI	5-15 mg/kg/day every 12 hr (PO)	Hypertrichosis, fluid overload* neutropenia, thrombocytopenia, respiratory distress, hyperglycemia
Octreotide	HI	2-20 mcg/kg/day every 6 hr (SQ)	Gallstones, transaminitis, malabsorption, suppression of thyroid and growth hormones
Lanreotide	HI	60-90 mg every 28 days (SQ)	Gallstones, transaminitis, malabsorption, suppression of thyroid and growth hormones
Enteral D20	HI	Up to 10 mg/kg/min continuously (via gastrostomy or nasogastric tube)	Vomiting, diarrhea, fluid overload
Cornstarch	GSD, rarely FAOD, IKH	1-2 g/kg per dose (PO)	Diarrhea in infants
Growth hormone	Growth hormone deficiency	0.3 mg/kg/wk daily or every 12 hr (infants) (SQ)	Pseudotumor cerebri, edema, slipped capital femoral epiphysis, hyperglycemia
Hydrocortisone	Adrenal insufficiency	8-12 mg/m^2/day (PO)	High doses: Immunosuppression, hyperglycemia, hypertension, obesity
Acarbose	Postprandial hypoglycemia	12.5-50 mg with meals	Transaminitis, diarrhea, abdominal pain
PANCREATECTOMY			
Partial	Focal HI Insulinoma		>50% pancreatectomy increases risk of diabetes and exocrine insufficiency later in life
Near-total (98%)	Severe diffuse HI		Insulin-dependent diabetes, exocrine insufficiency

*Concomitant use of diuretics is strongly recommended in neonates and infants; older children may require this as well.
HI, Hyperinsulinism; GSD, glycogen storage disease; FAOD, fatty acid oxidation disorder; IKH, idiopathic ketotic hypoglycemia.

Table 113.8 Criteria for Diagnosing Hyperinsulinism and Other Forms of Insulin Excess

DRAWN AT A TIME OF FASTING HYPOGLYCEMIA: PLASMA GLUCOSE <50 MG/DL
Detectable insulin or c-peptide (plasma insulin ≥2 μU/mL or plasma C-peptide ≥0.5 ng/mL)*
Hypofattyacidemia (plasma free fatty acids <1.7 mmol/L)
Hypoketonemia (plasma β-hydroxybutyrate <1.8 mmol/L)
Inappropriate glycemic response to glucagon (increase in glucose >30 mg/dL)

*Depends on sensitivity of insulin assay. Detectable insulin and C-peptide is not necessary to make a diagnosis of HI.

tachyphylaxis. A long-acting analog, lanreotide, is administrated monthly in children greater than 1 year old and can be an effective and more convenient alternative to octreotide. Somatostatin analogs should not be used in neonates or infants less than 2 months old because they are associated with fulminant necrotizing enterocolitis. Continuous intragastric dextrose administered via gastrostomy tube is used in combination with a somatostatin analog and allows for an age-appropriate feeding schedule.

Infants Born to Diabetic Mothers

See Chapter 147.1.

Gestational diabetes affects approximately 2% of pregnant women, and 1 in 1,000 pregnant women have insulin-dependent diabetes. Infants born to mothers with poorly controlled diabetes are born large for gestational age and with severe hypoglycemia resulting from a transient hyperinsulinemic state. Exposed to high glucose concentrations, the fetal islets compensate with increased insulin secretion that, during intrauterine life, leads to overgrowth and, after birth, results in hypoglycemia. The hypoglycemia resolves within the first 3-7 days of life. Mothers whose diabetes has been well controlled during pregnancy, labor, and delivery generally have infants near normal size who are less likely to develop hypoglycemia.

Insulinoma

Older children and adolescents presenting with insulin-mediated hypoglycemia should be evaluated for an **insulinoma**, a rare islet cell tumor. These tumors may be insidious, slow growing, and difficult to localize with conventional imaging modalities. Surgical resection is curative. Children diagnosed with an insulinoma should undergo genetic testing for the tumor predisposition syndrome, multiple endocrine neoplasia type 1 (MEN1).

Factitious Hypoglycemia

Surreptitious administration of insulin presents with abrupt onset and erratic hypoglycemia. In a younger child, a parent or guardian may provoke hypoglycemia (factitious disorder imposed on another: Munchhausen by proxy), but an adolescent may be self-administering insulin. The biochemical hallmark of exogenous insulin administration is a detectable insulin level with undetectable c-peptide. However, specialized assays may be needed to detect the analog insulins, such as aspart or glargine. Intentional or accidental ingestion of sulfonylureas also presents with hypoglycemia. In these cases, both insulin and c-peptide are detectable, and sulfonylurea blood levels are required for confirmation.

Defects of Fatty Acid Oxidation

Fatty acid oxidation disorders are characterized by low plasma β-hydroxybutyrate and high plasma FFAs. See Chapter 106.1.

Multiple enzymatic deficiencies in the mitochondria pathway of fatty acid oxidation cause defective carnitine or fatty acid metabolism. These disorders are characterized by fasting hypoglycemia, hepatomegaly, cardiomyopathy, and hypotonia, although the spectrum of severity is wide. Infants may present with a Reye-like syndrome (see

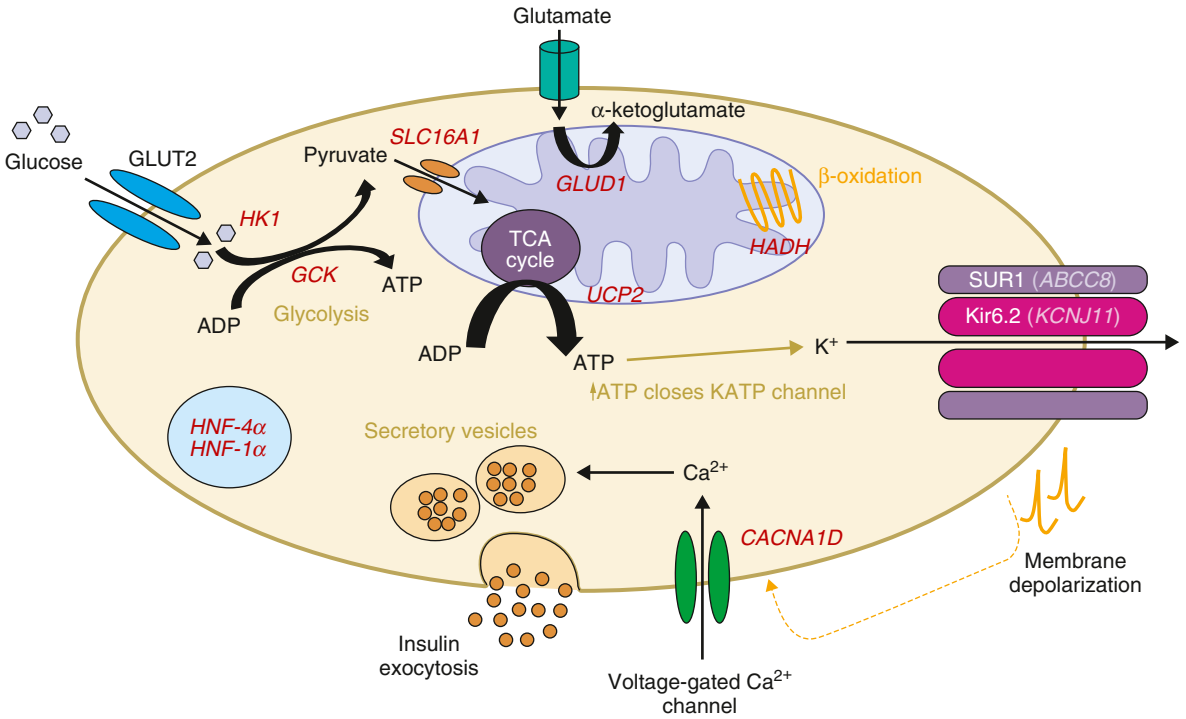

Fig. 113.5 Diagrammatic representation of β-cell function. Genetic defects associated with congenital HI are included in *red*. Postprandial glucose is taken into the β-cells via the glucose transporter 2 (GLUT-2). Glucose then enters the glycolysis pathway followed by the mitochondrial citric acid cycle (TCA), yielding the high-energy molecule, adenosine triphosphate (ATP). ATP molecules travel to and inhibit the potassium-dependent ATP channels (K_{ATP}), which prevents influx of potassium resulting in membrane depolarization. This triggers voltage-gated calcium channels to open, and influx of calcium (Ca^{2+}) occurs. The Ca^{2+} activates the enzyme phospholipase C (PLC) to produce inositol 1,3,5 triphosphate (IP3) and diacylglycerol (DAG) from phosphatidyl 1,3 bisphosphate (PIP2). The IP3 molecule binds to the protein receptor on the endoplasmic reticulum (ER) to promote a release of Ca^{2+} from the ER. This subsequent increase in cytoplasmic Ca^{2+} promotes exocytosis of the prepackaged mature insulin and active C-peptide, which are released into circulation. GLUT2: Glucose transporter 2; Glucokinase (GCK) encoded by GCK gene; ADP: Adenosine diphosphate; ATP: Adenosine triphosphate; Monocarboxylate transporter (MCT1) encoded by *SLC16A1* gene; Glutamate dehydrogenase (GDH) encoded by *GLUD1* gene; Uncoupling protein 2 (UCP2) encoded by *UCP2* gene; L-3hydroxyacyl-coenzyme A dehydrogenase (HADH) encoded by *HADH* gene; SUR1 subunit of the K_{ATP} channel encoded by the *ABCC8* gene; Kir6.2 subunit of the K_{ATP} channel encoded by *KCNJ11* gene; Hepatocyte nuclear factor 4α (HNF4α) encoded by *HNF4A* gene; Hepatocyte nuclear factor 1α (HNF1α) encoded by *HNF1A* gene; HK1: Hexokinase 1 encoded by the gene *HK1*; CACNA1D: calcium voltage-gated channel subunit alpha1 D. Gene variants in forkhead box protein A2 (FOXA2), phosphoglucomutase 1 (PGM1), and phosphomannomutase 2 (PMM2) are not included in the drawing. (*From Güemes M, Rahman SA, Kapoor RR, et al. Hyperinsulinemic hypoglycemia in children and adolescents: recent advances in understanding of pathophysiology and management. Rev Endo Metab Dis. 2020;21:577–597, Fig. 1.*)

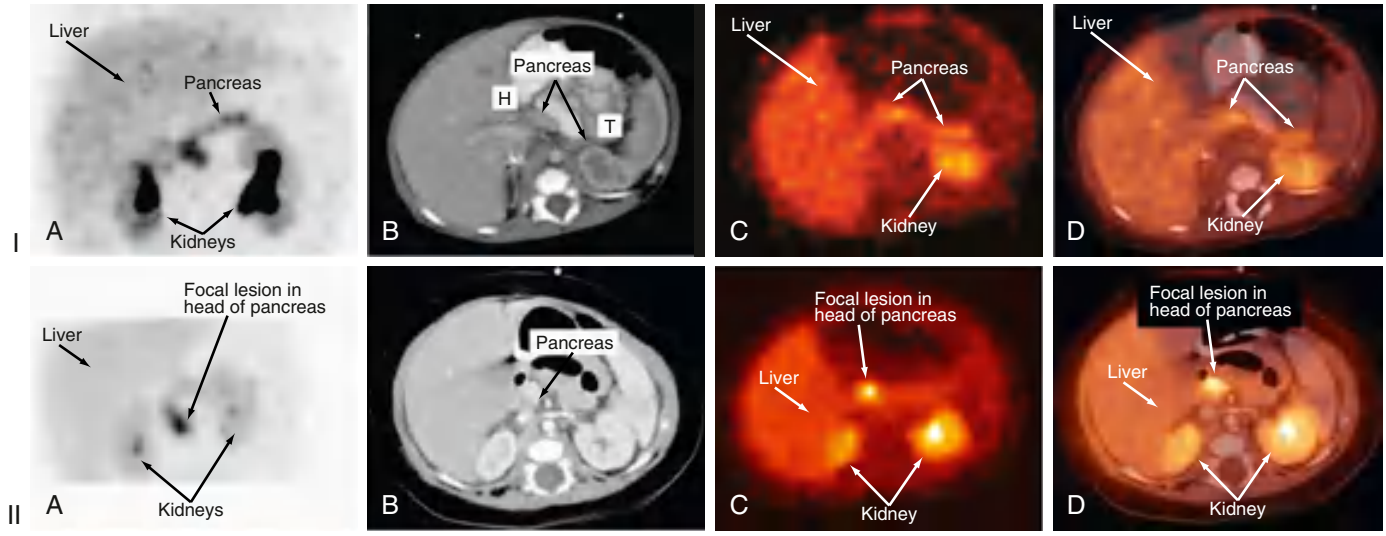

Fig. 113.6 Imaging of congenital hyperinsulinism. **I** Panels *(diffuse)*: 18-fluoro L-3,4-dihydroxyphenylalanine positron emission tomography (^{18}F-DOPA PET) of a patient with a diffuse form of congenital hyperinsulinism. **A,** Diffuse uptake of ^{18}F-DOPAa is visualized throughout the pancreas. Transverse views show **(B)** normal pancreatic tissue on abdominal CT, **(C)** diffuse uptake of ^{18}F-DOPA in the pancreas, and **(D)** confirmation of pancreatic uptake of ^{18}F-DOPA with co-registration. H, Head of pancreas; T, tail of pancreas. **II** Panels *(focal)*: ^{18}F-DOPA PET of a patient with a focal form of congenital hyperinsulinism. **(A)** Discrete area of increased ^{18}F-DOPA uptake is visualized in the head of the pancreas. The intensity of this area is greater than that observed in the liver and neighboring normal pancreatic tissue. Transverse views show **(B)** normal pancreatic tissue on abdominal CT, **(C)** focal uptake of ^{18}F-DOPA in the pancreatic head, and **(D)** confirmation of ^{18}F-DOPA uptake in the pancreatic head with co-registration. (*Courtesy Dr. Olga Hardy, Children's Hospital of Philadelphia.*)

Fig. 113.7 Beckwith-Wiedemann syndrome. (*Courtesy Dr. Michael Cohen, Dalhousie University, Halifax, Nova Scotia. From Jones KL. Smith's recognizable patterns of human malformation, 6th ed. Philadelphia: Saunders; 2006.*)

Chapter 409) and recurrent episodes of fasting hypoglycemia, cardiorespiratory arrest, and coma, whereas older children with less severe forms may only develop symptoms with illness. Most cases are detected via newborn screening and confirmed with genetic testing. Abnormalities are seen in the acylcarnitine and urine organic acid profiles in children who are not identified through newborn screening. Treatment typically involves avoidance of fasting and administration of dextrose-containing fluids with illness.

Interference with fatty acid metabolism also underlies the fasting hypoglycemia associated with Jamaican vomiting sickness. In **Jamaican vomiting sickness**, the unripe ackee fruit contains a water-soluble toxin, hypoglycin, which produces vomiting, CNS depression, and severe hypoglycemia. The hypoglycemic activity of hypoglycin derives from its inhibition of gluconeogenesis secondary to its interference with the acyl-CoA and carnitine metabolism essential for the oxidation of long-chain fatty acids. The disease is almost totally confined to Jamaica, where ackee forms a staple of the diet. The ripe ackee fruit no longer contains this toxin. A similar illness noted in India, **acute toxic encephalopathy-hypoglycemic syndrome**, may be caused by litchi consumption. Litchi contains hypoglycin A and/or methylenecyclopropylglycine, which may inhibit fatty acid oxidation or gluconeogenesis.

KETOTIC HYPOGLYCEMIC DISORDERS

These disorders are characterized by elevated levels of plasma β-hydroxybutyrate (>2.5 mmol/L) (Table 113.9).

Glycogen Storage Disorders

See Chapter 107.1.

The **hepatic glycogen storage disorders** (GSDs; 0, I, III, VI, and IX) result from genetic defects in the enzymes that regulate the synthesis, breakdown, and release of glycogen in the liver. GSD type I also affects gluconeogenesis. GSDs are characterized by fasting ketotic hypoglycemia and various degrees of hepatomegaly, liver function abnormalities, hyperlipidemia, and

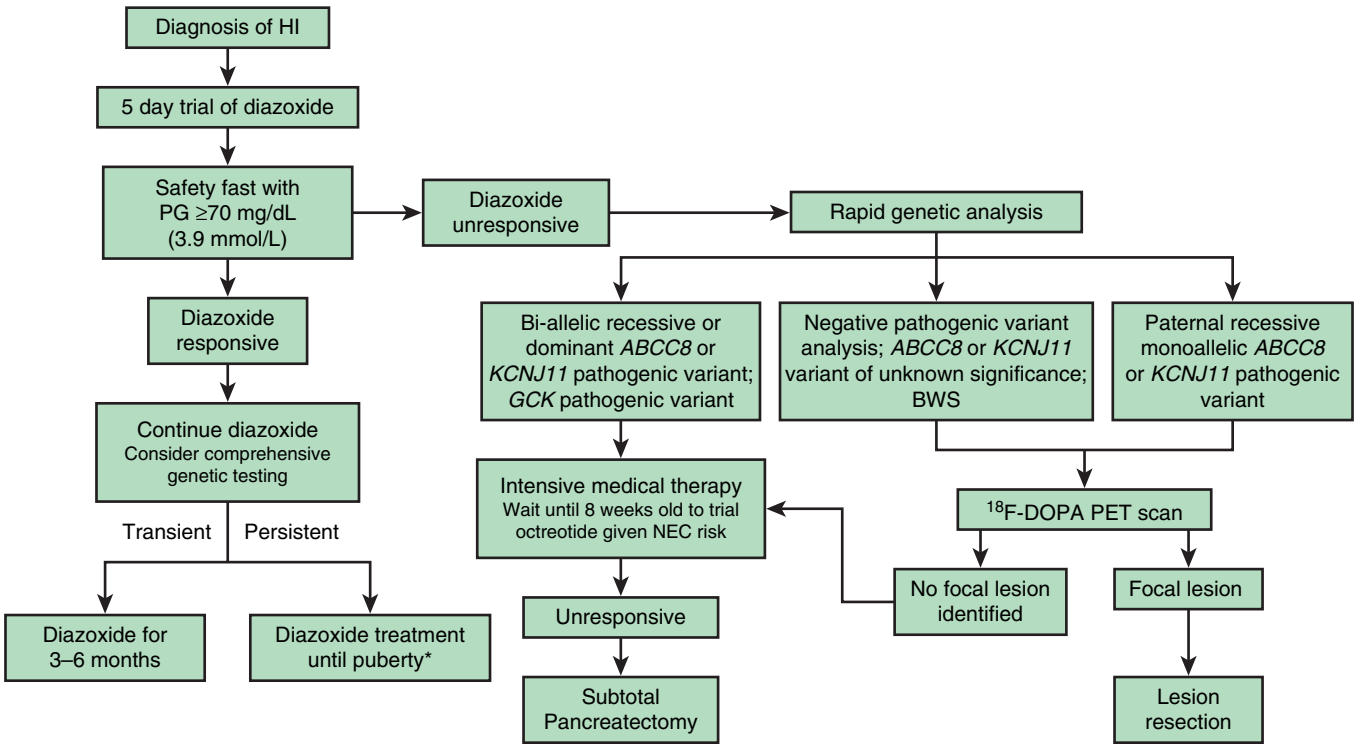

Fig. 113.8 Management algorithm for hyperinsulinism. HI, Hyperinsulinism; BWS, Beckwith-Wiedemann syndrome. *Some individuals may require diazoxide treatment into adulthood and possibly lifelong.

Table 113.9 Causes of Ketotic Hypoglycemia in Children

	GENE OR CHROMOSOME	INHERITANCE
HORMONAL		
Growth hormone deficiency or resistance	Genetic or acquired	Variable
ACTH deficiency or resistance; cortisol deficiency	Genetic or acquired	Variable
Glucagon deficiency*	*GCG, DBH*	N/D
Dopamine β-hydroxylase deficiency*	*GCG, DBH*	N/D
METABOLIC		
Glycogen storage disease (GSD)		
GSD 0; glycogen synthase deficiency	*GYS2*	AR
GSD III; glycogen debranching enzyme deficiency	*AGL*	AR
GSD VI; glycogen phosphorylase deficiency	*PYGL*	AR
GSD IX; phosphorylase kinase subunit deficiencies	*PHKA2, PHKB, PHKG2*	X-linked, AR
GLUCOSE METABOLISM AND TRANSPORT		
Phosphoglucomutase I deficiency	*PMG1*	AR
Pyruvate carboxylase deficiency	*PC*	AR
ORGANIC ACIDEMIAS		
Maple syrup urine disease, propionic aciduria, methylmalonic aciduria	Multiple genes	AR
Ketone body transport and metabolism		
Monocarboxylase transporter 1 defect	*SLC16A1 (MCT1)*	AR, AD
Ketolysis		
Succinyl CoA oxoacid transferase deficiency	*SCOT*	AR
Mitochondrial acetoacetyl-CoA thiolase (β-ketothiolase) deficiency	*ACAT1*	AR
SYNDROMES		
Silver-Russel syndrome	11p15 or 7**	Mostly sporadic
Prader-Willi syndrome	15q11-q13***	Mostly sporadic
Fanconi-Bickel syndrome	*SLC2A2 (GLUT2)*	AR
KH secondary to chronic malnutrition, severe malaria, other chronic diseases	—	—
Idiopathic ketotic hypoglycemia		
Physiologic KH in prolonged fasting or acute illness	—	—
Pathologic KH	—	—
IGF2BP1 deficiency*	*IGF2BP1*	N/D
Sodium glucose co-transporter 2 defect*	*SLC5A2*	N/D
PEP carboxykinase 1 and G-6P catalytic transcriptional induction*	*NCOR1*	N/D
Mitosis gene A-related kinase 11 defect*	*NEK11*	N/D

*Suggested, not well-established, causes of KH.
**Several mechanisms, rare other mechanisms, or unknown.
***Paternal deletion, maternal uniparental disomy, or imprinting defect.
AD, Autosomal dominant; AR, autosomal recessive; N/D, no data; PEP, phosphoenolpyruvate; G-6P, glucose 6-phosphate. The list is not fully inclusive.
From Drachmann D, Hoffman E, Carrigg A, et al. Towards enhanced understanding of idiopathic ketotic hypoglycemia: a literature review and introduction of the patient organization, Ketotic Hypoglycemia International. *Orphanet J Rare Dis*. 2021;16:173, Table 1.

failure to thrive. Muscle involvement is a feature of GSD type III, resulting in proximal muscle weakness and risk of cardiomyopathy. GSD types 0, VI, and IX present with less severe hypoglycemia compared with type I and III. The diagnosis of GSD is suspected in patients presenting with ketotic hypoglycemia, hepatomegaly, and liver enzyme abnormalities and is confirmed through genetic testing. The treatment of GSDs consists of uncooked cornstarch therapy, protein supplementation, and limited fasting.

Hormone Deficiencies

Deficiencies of cortisol or growth hormone can result in ketotic hypoglycemia, as these counter-regulatory hormones play a role in mobilizing glucose production by the liver. Hypoglycemia occurs in both primary or secondary (central) **adrenal insufficiency** and is commonly seen during an adrenal crisis. **Growth hormone deficiency** results in hypoglycemia mainly during infancy and early toddlerhood. Combined hormone deficiencies, known as hypopituitarism, can also result in hypoglycemia and are commonly caused by structural abnormalities of the pituitary gland. A clinical clue to the diagnosis of hypopituitarism is the presence of midline defects, cleft lip or palate, or in males, a microphallus. In the neonatal period, hypopituitarism presents with a hypoketotic hypoglycemia pattern that is indistinguishable from the pattern seen in neonates with HI. Thus in neonates with features suggestive of hypopituitarism, it is important to rule out hormone deficiencies before making a diagnosis of HI.

Low cortisol and growth hormone levels on a critical-sample laboratory result are suggestive of hormone deficiencies but not diagnostic. To confirm these diagnoses in a child presenting with hypoglycemia, appropriate hormone stimulation testing is recommended. A child diagnosed with central adrenal insufficiency, growth hormone deficiency, or hypopituitarism requires magnetic resonance imaging of their pituitary gland to assess for structural abnormalities or lesions. Hormone replacement with either hydrocortisone or growth hormone

resolves the hypoglycemia. Neonates and infants require twice-a-day growth hormone dosing to prevent ongoing hypoglycemia.

Idiopathic Ketotic Hypoglycemia

Idiopathic ketotic hypoglycemia (IKH) commonly occurs during the first 1-4 years of life and typically resolves by age 6-8 years old. Children present with hypoglycemia and elevated β-hydroxybutyrate (>2-3 mmol/L), frequently in the setting of illness, and demonstrate shortened fasting tolerance on a diagnostic fast. IKH is a diagnosis of exclusion, and it is important to rule out other causes of ketotic hypoglycemia, such as GSDs or hormone deficiencies, before making this diagnosis. IKH is likely the result of the lower energy stores in toddlers and young children and is not caused by an underlying metabolic defect, as evidenced by its spontaneous resolution (see Table 113.9). Treatment typically consists of limited fasting time and home glucose monitoring during times of illness. IV dextrose infusion may be required with illness if children are unable to tolerate oral feeds.

Disorders of Gluconeogenesis

Disorders of gluconeogenesis are characterized by elevated plasma lactate levels during hypoglycemia.

Glycogen Storage Disease Type I

See Chapter 107.1.

Glucose-6-phosphatase regulates the terminal step of gluconeogenesis and glycogenolysis, and deficiency of this enzyme results in GSD type I. Children with this condition experience hypoglycemia within 2-3 hours after a meal and significant elevations of lactate, uric acid, and triglycerides because of shunting of gluconeogenic precursors to alternative pathways in the liver. The diagnosis should be suspected in an infant with hypoglycemia, hepatomegaly, and the characteristic metabolic abnormalities. Sequencing of *G6PC* (type Ia) or *SLC37A4* (type Ib) confirms the diagnosis. Treatment includes frequent meals or feeds and after the first 6-12 months of life, uncooked cornstarch administered every 3-4 hours. Fasting beyond 4-6 hours can provoke life-threatening lactic acidosis.

Fructose-1,6-Bisphosphatase Deficiency

See Chapter 107.3.

A deficiency of this enzyme results in a block of gluconeogenesis from all possible precursors below the level of fructose-1,6-bisphosphate. Features are similar to GSD type I with fasting hypoglycemia, severe lactic acidosis, and elevations of uric acid and triglycerides. However, glycogenolysis remains intact, so patients do not have significant hepatomegaly or transaminitis. The diagnosis is confirmed through genetic testing of *FBP1*. Similar to GSD type I, treatment involves frequent meals, cornstarch, and avoidance of prolonged fasting.

OTHER CAUSES OF HYPOGLYCEMIA

Postprandial Hypoglycemia

Up to 30% of infants and children with **Nissen fundoplication**, a procedure used to ameliorate gastroesophageal reflux, develop **postprandial hypoglycemia**, or "late dumping syndrome." Characteristic features include hyperglycemia followed by severe hypoglycemia (average 32 mg/dL in one series) 1.5-3 hours later. The early hyperglycemia phase is associated with brisk and excessive insulin release that causes the resultant hypoglycemia. A role for exaggerated GLP-1 secretion has been proposed as being responsible for the excessive insulin release. Treatment consists of feed modifications for formula- or tube-fed children, such as the use of complex formulas or prolonged feeds. Acarbose has been successfully used in some orally fed children.

Acute Alcohol Intoxication

The generation of reducing equivalents during the oxidation of ethanol in the liver inhibits several gluconeogenic enzymes, resulting in hypoglycemia if glycogen stores are depleted by starvation or by preexisting abnormalities in glycogen metabolism. There is no correlation between blood ethanol levels and the occurrence of hypoglycemia. In toddlers who have been fasting for some time, even the consumption of small quantities of alcohol can precipitate hypoglycemia. *Alcohol-induced hypoglycemia promptly responds to IV dextrose.* However, if there is an associated alcohol toxicity, the patient may not show clinical signs of improvement despite now having a normal blood glucose level. A careful history allows the diagnosis to be made and may avoid additional workup.

β Blockers

β-Adrenergic stimulation enhances glucagon secretion and increases glycogenolysis and gluconeogenesis. β blockers impair the counterregulatory response to hypoglycemia and blunt the autonomic symptoms described earlier. Children at highest risk of hypoglycemia from β blockers include those with insulin-dependent diabetes and infants and toddlers in the setting of prolonged fasting or illness. Despite increased use of propranolol for infantile hemangiomas, the prevalence of hypoglycemia in this population appears to be low (0.9%). Propranolol has also been safely used in children with underlying hypoglycemia disorders, although close monitoring of glucose is needed during illness.

Salicylate Intoxication

See Chapter 94.

Both hyperglycemia and hypoglycemia occur in children with salicylate intoxication. Accelerated use of glucose, resulting from augmentation of insulin secretion by salicylates, and possible interference with gluconeogenesis may contribute to hypoglycemia. Infants are more susceptible than older children. Monitoring of blood glucose levels with appropriate glucose infusion in the event of hypoglycemia should form part of the therapeutic approach to salicylate intoxication in childhood. Ketosis may occur.

Systemic Disorders

Several systemic disorders are associated with hypoglycemia in infants and children. Children with acute liver failure develop hypoglycemia because of loss of gluconeogenesis. Sepsis, particularly in neonates, may result in hypoglycemia. Falciparum malaria is also associated with hypoglycemia, which is thought to be the result of increased glucose use and impaired gluconeogenesis.

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