CHAPTER

43

Inborn Metabolic Defects of Lysosomes, Peroxisomes, Carbohydrates, Fatty Acids and Mitochondria

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OUTLINE

LYSOSOMAL STORAGE DISEASES

The cell contains specialized organelles for the recycling of waste material: the lysosomes

About 60 years ago, Belgian cytologist Christian de Duve discovered that animal cells contained small 0.1–1.2μm organelles limited by a single membrane and containing acidic hydrolases, active at pH ~4.5. The lysosome (from the Greek *lysis*, to separate and *soma*, body) is the subcellular organelle responsible for the physiological turnover of cellular debris, waste compounds, aged organelles, bacteria and viruses.

Lysosomal enzymes are synthesized in the endoplasmic reticulum, transported through the Golgi apparatus and

post-translationally modified by glycosylation and the addition of mannose-6-phosphate tags. Mannosylation is needed for proper binding to trans-Golgi network mannose-6-phosphate receptors (M6PR), which help deliver lysosomal enzymes to pre-lysosomes (Hickman & Neufeld, 1972; Hickman et al., 1974). Lysosomal enzymes are primarily targeted to the lysosomal compartment, but a fraction can follow the secretory route and be released in the extracellular milieu. Plasma membrane–bound M6PR mediates the internalization of extracellular lysosomal enzymes via the clathrin-endocytic pathway.

Deficiency of a lysosomal enzyme causes the blockage of the corresponding metabolic pathway, leading to the accumulation of its undigested substrate

Lysosomal storage diseases encompass at least 45 entities with an overall estimated incidence of ~1:8,000 (Meikle et al., 1999). Twenty-nine of these diseases involve defects in genes that code for acid hydrolases (Table 43-1). They generally have an autosomal-recessive mode of inheritance, except

TABLE 43-1 Lysosomal Storage Diseases

PRIMARY LYSOSOMAL HYDROLASE DEFECTS		
Name	**Enzyme defect**	**Stored substrate**
Farber disease	Acid ceramidase	Ceramide
Gaucher disease	Glucosylceramidase	Glucosylceramide Glucosphingosine
Krabbe disease	Galactosylceramidase	Galactosylceramide Galactosylsphingosine
Metachromatic leukodystrophy	Arylsulfatase A	Sulfatides
Fabry disease	α-galactosidase	Trihexosylceramide (gal-gal-glc-cer, digalactosylceramide)
GM1-gangliosidosis, Morquio B	Acid β-galactosidase	GM1 ganglioside, Keratan sulfate
GM2-gangliosidose - Tay–Sachs disease - Sandhoff disease	 Hexosaminidase A Hexosaminidases A and B	 GM2-ganglioside asialo-GM2-ganglioside, globoside
Niemann–Pick A and B	Acid sphingomyelinase	Sphingomyelin
Hurler syndrome MPS1H Scheie syndrome MPS1S	Alpha-iduronidase	Dermatan sulfate Heparan sulfate
Hunter syndrome MPS 11	Iduronate sulfatase	Dermatan sulfate Heparan sulfate
Sanfilippo A MPS IIIA	Heparan-N sulfatase	Heparan sulfate
Sanfilippo B MPS IIIB	N-acetyl-glucosaminidase	Heparan sulfate
Sanfilippo C MPS IIIC	Acetyl CoA glucosamine N-acetyl transferase	Heparan sulfate
Sanfilippo D MPS IIID	N-acetyl-glucosamine 6-sulfatase	Heparan sulfate
Morquio A MPS IVA	N-acetyl-galactosamine 6-sulfatase	Keratan sulfate
Maroteaux–Lamy MPS VI	N-acetyl-galactosamine-4-sulfatase	Dermatan sulfate
Sly syndrome MPS VII	β-glucuronidase	Heparan sulfate Dermatan sulfate
Hyaluronidase deficiency	Hyaluronidase	Hyaluronic acid
Fucosidosis	Fucosidase	Fucoside glycolipids
α-mannosidosis	α-mannosidase	α-mannosides
β-mannosidosis	β-mannosidase	β-mannosides
Aspartylglucoaminuria	Aspartyl-glucosaminidase	Aspartyl-glucosamine
Schindler disease	α-galactosidase-B	N-acetyl-galactosamide glycolipids
Sialidosis; mucolipidosis I	α-neuraminidase	Sialic acid
Pompe disease	α-glucosidase	Glycogen

(Continued)

TABLE 43-1 (Continued)

Name	Enzyme defect	Stored substrate
Wolman disease	Acid lipase	Cholesterol ester
Infantile neuronal ceroid lipofuscinoses CLN1 or PPT1	Palmitoyl protein thioesterase	Saposins
Late infantile neuronal ceroid lipofuscinoses CLN2	Carboxypeptidase	Subunit C mitochondrial ATP synthase
EXAMPLES OF OTHER TYPES OF LYSOSOMAL STORAGE DISEASES WITHOUT PRIMARY DEFECTS IN LYSOSOMAL HYDROLASES		
Multiple sulfatase deficiency	All sulfatases	Multiple mucopolysaccharides, oligosaccharides and glycoproteins
Mucolipidosis type II and III	Phosphotransferase-mediated phosphorylation of mannoses	Multiple compounds
Mucolipidosis type IV	MCOLN1	Multiple compounds
Galactosialidosis	β-galactosidase and neuraminidase	Sialyloligosaccharides
Niemann–Pick disease C	NPC1	Unesterified cholesterol in liver & spleen
G_{M2} activator protein deficiency	G_{M2} activator protein	G_{M2} and G_{A2}
Sphingolipid activator protein deficiency	Saposins	Multiple sphingolipids
Danon disease (LAMP-2 deficiency)	LAMP2	glycogen

Source: Adapted from reference (Platt & Walkley, 2004)

for Hunter and Fabry diseases, which are X-linked recessive traits. The defective genes have been identified and mutations have been defined for nearly all. The nervous system is involved in most of these diseases, although lysosomes are ubiquitous. Many of the disorders develop in early infancy, but adolescent and adult variants are also present in many diseases. Clinical severity ranges from death in early childhood to moderate disability in adulthood. Diagnosis of lysosomal disorders is usually based on enzymatic assays in white blood cells or in cultured skin fibroblasts (Scriver et al., 2001).

For most lysosomal storage diseases, definitive cures are not available

Available treatments based on enzyme replacement may lead to stabilization and even partial regression in some patients. Research on mechanisms that regulate the genesis and transport of lysosomal enzymes has improved cell transplantation and enzyme replacement therapies. Transplantation has focused primarily on use of bone marrow–derived and cord blood–derived hematogenous stem cells isolated from HLA-compatible healthy donors. The rationale is that engrafted donor cells will generate healthy hematogenous lineages. Donor-derived monocytic cells generate macrophages, which appear to infiltrate the nervous system and contribute to endogenous microglia (Krivit et al., 1995). Although microglial replacement is incomplete, it increases normal enzyme activity in host nervous tissue through enzyme secretion and reuptake (cross-correction). This strategy is clinically effective when applied to asymptomatic newborns or certain late-onset cases, but patients with neurological symptoms do not benefit.

Lysosomal storage disorders are pleiotropic, depending on the mutation, the enzyme affected and the sites of accumulated products

The following sections describe representative diseases in more detail. Detailed description of all lysosomal disorders is found in standard reference volumes (Scriver et al., 2001; Platt et al., 2004).

Farber disease

Farber disease is caused by deficiency of acid ceramidase and storage of undegraded ceramides, particularly ceramides containing 2-hydroxy fatty acids, in lysosomes (Sugita et al., 1972). Gangliosides can also accumulate. Clinical manifestations of the disease occur commonly during the first months of life with deformation of joints, progressive hoarseness, granulomatous infiltration of subcutaneous tissues, kidney and lungs. Neurological deficiencies occur frequently. Transplantation of hematopoietic cells is a promising strategy to prevent deterioration, especially in cases without major neurological complications (Vormoor et al., 2004). *In vitro* and *in vivo* gene transfer experiments also proved that Farber disease is a good candidate for gene therapy (Medin et al., 1999; Ramsubir et al., 2008).

Gaucher disease

Gaucher disease is caused by deficiency of glucosylceramidase with storage of glucosylceramide and glucosylsphingosine (glucopsychosine). The most common form of the disease, Gaucher type I, does not involve the nervous system and manifests with enlarged liver and spleen and

abnormal bone marrow, leading to anemia, thrombocytopenia and skeletal changes. Gaucher type I can have childhood or adult onset and survive up to 80 years. Gaucher type II is compounded by severe damage to the nervous system: it manifests soon after birth and leads to death in early childhood. Gaucher type III is intermediate: patients develop nervous system abnormalities in adolescence, surviving into their forties.

Accumulation of glucopsychosine, a potent neurotoxic lysosphingolipid, may play an important role in pathogenesis of Gaucher disease (Miyatake & Suzuki, 1973; Conradi et al., 1984). Most mutations are missense, leading to enzymes with lower activity. The most common mutation, N370S, is found only in Gaucher type 1 but never in association with a neuropathic phenotype. Mutation L444P is present in neuropathic forms of the disease. Enzyme replacement ameliorates most clinical symptoms in Gaucher type I (Andersson et al., 2008); hematogenous stem cell transplantation works best in Gaucher type III (Ringden et al., 1995). Substrate reduction and gene transfer therapies also are promising, as well as chemical chaperone therapy (Zheng et al., 2007).

Krabbe disease (globoid cell leukodystrophy)

Krabbe disease (globoid cell leukodystrophy) is a demyelinating lysosomal storage disease caused by deficiency in galactosylceramidase with accumulation of galactosylceramide and galactosylsphingosine (psychosine). Psychosine is a potent neurotoxic lysosphingolipid (Igisu & Suzuki, 1984; Suzuki, 1998) that associates primarily with cholesterol-enriched lipid raft domains in plasma membrane (White et al., 2009; White et al., 2011) and is thought to cause apoptotic cell death of myelinating oligodendroglia (Jatana et al., 2002; Zaka & Wenger, 2004). Because of its subcellular location in lipid rafts (White et al., 2009), psychosine may exert harmful effects on multiple signaling pathways such as PKC (White et al., 2009; Yamada et al., 1996).

About 90% of cases have the infantile form, presenting before 6 months with general irritability, stiffness, and arrest of mental and motor development, progressing to a decerebrate state with no voluntary movements. Infantile cases die within 1–2 years. Juvenile and adult onset variants also exist.

The main histopathological change is extensive demyelination accompanied by astrogliosis, multinuclear globoid cells, axonopathy and defects in some neuronal populations (Scriver et al., 2001). By electron microscopy, undigested material is frequently seen as longitudinal crystals known as Krabbe crystals (Figure 43-1). The defective gene maps to 14q31. The 502T/del mutation is present in about 50% of patients. This mutation deletes about 30 kb downstream of intron 10 and eliminates the entire coding region for the 30 kDa subunit of galactosylceramidase and about 10% of the carboxy end of the 50 kDa subunit. Homozygous patients for 502T/del develop the severe infantile form and heterozygous cases are associated with adult forms. Mutation D270N is always found in adult onset patients.

Hematopoietic cell replacement is the best option for slowly progressive adult and early infantile cases without neurological deterioration (Krivit et al., 1995; Escolar et al., 2005; Krivit et al., 1998). Alternative approaches, such as gene transfer, enzyme therapy, substrate reduction and transplantation of neural stem cells are under active investigation. In Krabbe disease, as in other storage diseases with neurological involvement, the timing and distribution of enzyme for correction of the metabolic deficiency influence clinical success.

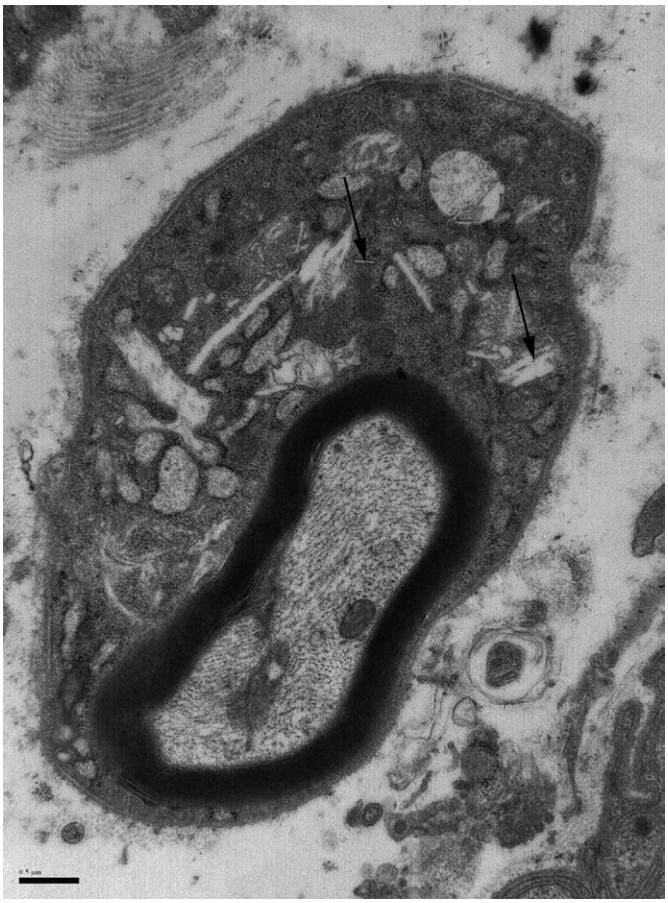

FIGURE 43-1 **Electron micrograph of a *Twitcher* myelinated axon**. The cross-section of this sciatic nerve was taken from a sick mutant mouse and shows an axon engulfed by its Schwann cell. The cytoplasm of the myelinating Schwann cell is filled with enlarged vacuoles and membrane structures. Crystallized deposits of undigested material, also known as Krabbe crystals, are clearly visible (arrows). Bar: 500 nm. (Courtesy of Robert P. Becker et al., College of Medicine, University of Illinois at Chicago).

Metachromatic leukodystrophy (MLD)

MLD is caused by mutations affecting activity of arylsulfatase A, a lysosomal enzyme responsible for degradation of sulfated galactolipids (Vos et al., 1994). Sulfatides account for about 4% of total lipid content of myelin. Sulfatides may have additional functions because they appear to participate in membrane dynamics, apparently by their association with lipid rafts (Vos et al., 1994) (see Box). Not surprisingly, myelinating glial cells are defective in this disease. This defect causes myelin loss and reduces the numbers of oligodendrocytes in white matter tracts, producing deposits of metachromatic granules, which stain red with basic dyes like cresyl

violet due to the anionic sulfate group of the stored material. Studies in a mouse animal model (Hess et al., 1996) allowed exploration of novel mechanisms that might explain the storage of sulfatide at the cellular level. Another animal model with accumulation of sulfatides primarily in neurons has been recently generated and opens new avenues of research (van Zyl et al., 2010).

There are different forms of the disease based on age at onset. Late infantile and juvenile cases comprise about 80% of patients. The infantile form is the most severe variant with clinical signs appearing early in life (15–24 months). Affected children have impaired walk, reduced speech and defective vision, and, die within a few years of onset. The juvenile form (occurring at 4–12 years) starts with poor performance at school. Seizures are common. Most juvenile cases do not live into adulthood. The adult form begins late in adolescence or in early adulthood with problems in thinking, reasoning and memory, often mimicking psychosis or dementia. MLD patients with the late-onset form survive for several decades.

Currently, there is no effective treatment for MLD. Asymptomatic or mildly symptomatic forms may be stabilized by bone marrow or cord blood transplantation as evidenced by absence of neurological deterioration and slow progression (Krivit et al., 1999; Peters & Steward, 2003; Matzner et al., 2002). Gene therapy (Matzner et al., 2000; Consiglio et al., 2001; Luca et al., 2005) and cell replacement therapy using diverse cell sources (Matzner et al., 2002; Givogri et al., 2006; Givogri et al., 2008; Biffi et al., 2004) are promising strategies. Enzyme therapy may be beneficial, although difficulties to reach the nervous system are expected (Matzner et al., 2005).

Fabry disease

Fabry disease is an X-linked recessive disease caused by α-galactosidase A deficiency, leading to systemic accumulation of glycosphingolipids with α-galactosyl modifications. The storage material is primarily composed of globotriaosylceramide and galabiosylceramide as well as various α-galactosyl-blood group B neutral glycosphingolipid antigens (Schibanoff et al., 1969; Miyatake, 1969). Affected males show widely distributed intralysosomal accumulations of crystalline glycosphingolipids (Maltese crosses) in endothelium and smooth muscle from blood vessels.

Fabry disease is a panethnic disorder with an estimated incidence of 1:40,000, manifesting in male hemizygotes in childhood or adolescence. Recurring episodes of excruciatingly severe burning pain in fingers and toes, referred to as acroparesthesias, are the most distressing symptom. Other manifestations are angiokeratomas and characteristic opacities in cornea and lens. Progressive renal failure and ischemic strokes occur in adulthood, and are the leading cause of death in untreated males. Neurological involvement has been described in some patients, (Grunnet & Spilsbury, 1973; Sung, 1979; Dutsch & Hilz 2010). Approximately 50% of heterozygous women have milder corneal dystrophy and acroparesthesias in middle age or later.

Enzyme therapy, combined with palliative treatments including neuropathic pain drugs and blood pressure control, ameliorates kidney and heart dysfunction and improves quality of life (Brady, 2006; Lidove et al., 2007). Other emerging promising therapies, which may be used in combination with enzyme therapy, include inhibition of glucosylceramide synthase to reduce the synthesis of α-galactosyl-glycosphingolipids and thus decrease lysosomal storage (Dwek et al., 2002), and chemical chaperone therapy to assist in the folding, maturation and trafficking of mutant α-galactosidase A proteins to lysosomes (Fan & Ishii, 2007).

G_{M2} gangliosidoses (Tay–Sachs disease; Sandhoff disease and G_{M2} activator deficiency)

G_{M2} gangliosidoses are caused by mutations affecting activity of hexosaminidase A (Tay-Sachs), hexosaminidase A or B (Saravanan et al., 2004) or the activity of the G_{M2} activator protein GM2A (G_{M2} activator deficiency). These gangliosidoses are characterized by lysosomal accumulation of ganglioside G_{M2}, and other related glycolipids, including G_{A2}, G_{D2}, globosides, G_{D3} and G_{M3} (Scriver et al., 2001). Oligosaccharides with β-glycosyl N-acetylglucosamine residues also accumulate in Sandhoff disease.

Tay-Sachs is panethnic but occurs most frequently in the Ashkenazi Jewish population. The infantile form is most frequent. Affected children startle easily as newborns, appear listless, fail to follow objects visually at 3–6 months, and lose previously gained milestones. After 1 year, the child becomes increasingly immobile, with seizures and decerebrate posturing, and death occurs by 4–5 years.

The cherry-red macular spot, due to accumulation of gangliosides in retinal neurons except in the fovea, is a striking and characteristic finding. Juvenile variants and adult variants occur more rarely. Pathological studies show severe cortical atrophy and ballooned neurons that contain concentrically arranged electron-dense lamellar structures known as membranous cytoplasmic bodies (Terry & Korey, 1960; Samuels et al., 1963). These structures occur frequently within meganeurites near to axonal initial segments (Purpura & Suzuki, 1976). Secondary neurites branch from these abnormal processes, disrupting normal synaptic wiring. In late-onset forms of Tay-Sachs disease, neurological manifestations encompass a wide array of defects and may include dystonia, extrapyramidal signs such as ataxia and choreic movements, spinocerebellar degeneration, and motor neuron disease.

Both clinical symptoms and neuropathology of Sandhoff disease are similar to those of Tay–Sachs disease. G_{M2} activator deficiency was identified in a patient with Tay–Sachs phenotype, with massive storage of G_{M2} and G_{A2} in the brain despite presence of hexosaminidases A and B. This led to purification of an activator protein that is not itself catalytically active. It is a small lysosomal glycoprotein that 'lifts' membrane-bound G_{M2} ganglioside a few angstroms out of the membrane, increasing accessibility to degradative enzymes. The gene is located on chromosome 5 and five different mutations have been identified. The neuropathology of G_{M2} activator deficiency is identical to Tay-Sachs except for the presence of Zebra bodies in cortical neurons.

No effective treatment exists for infantile G_{M2} gangliosidoses. Enzyme therapy is promising but limited by inefficiency of enzymes crossing the blood–brain barrier (Desnick & Schuchman, 2002) (Chapter 1). Bone marrow transplants were ineffective in preventing neurological symptoms

(Hoogerbrugge et al., 1989), likely due to low secretion of hexosaminidase A from donor-derived microglia, but may be relevant to treat late-onset gangliosidoses. Substrate reduction therapies (Platt et al., 1997), gene therapy (Cachon-Gonzalez et al., 2006) and chemical chaperone therapy (Osher et al., 2010) are promising strategies under development.

Niemann–Pick disease, types A and B

Niemann–Pick diseases are due to a deficiency of acid sphingomyelinase. This leads to the accumulation of sphingomyelin in many tissues. Type A patients present in the first few months of life with greatly enlarged liver and spleen, hypotonia, muscular weakness and feeding difficulties (Scriver et al., 2001). The red-cherry macular spot is present in about half of type A cases. Type A patients lose motor function and develop spasticity and rigidity, and are increasingly disconnected from their surroundings. Clinical features in type B patients are more variable but differ strikingly from type A patients in that neurological function is generally normal. Both types develop pulmonary complications. At least 100 mutations affect enzyme activity (Schuchman, 2007). Histopathology shows monocytic cells filled with granules of lipid material (Niemann-Pick cells). These cells are virtually everywhere in late stages of the disease. In the nervous system, ganglion neurons are swollen with cytoplasmic lipid vacuoles. Myelin loss is significant. There is no specific therapy for Niemann-Pick. Bone marrow transplantation seems ineffective in type A (Bayever et al., 1992; Bayever et al., 1992), but may have better therapeutic potential when performed early in type B cases (Schneiderman et al., 2007; Shah et al., 2005).

Niemann–Pick disease type C (NPC)

NPC is caused by mutations in the NPC1 gene and to a lesser extent in the NPC2 gene (Vanier & Millat, 2003). The NPC1 gene maps to 18q11–12 and encodes a 170 kDa protein, which resides in endosomes. It is postulated that NPC1 regulates retrograde transport of multiple lysosomal cargoes in the late endosomal/lysosomal pathway (Chapter 8). NPC1 and NPC2 function cooperatively. NPC1 has homology to other proteins involved in cholesterol homeostasis, such as patched, HMG-CoA reductase and SCAP (Scriver et al., 2001). Impaired processing of endocytosed free cholesterol is the fundamental defect. Patients with the classical phenotype present progressive neurological disease in late infancy to adolescence, with clumsy gait, ataxia, school failure, behavior problems and a characteristic supranuclear downward gaze paralysis. There are varying degrees of hepatosplenomegaly. Infantile and adult-onset variants exist. In the brain, there is no evidence of cholesterol accumulation (Crocker, 1961) but glucosyl and lactosylceramides are significantly augmented. Neuronal inclusions are present throughout the nervous system. Unesterified cholesterol, sphingolipids and glycolipids are stored in excess in spleen and liver. Biochemical diagnosis of NPC depends on demonstration of a specific defect of cholesterol processing in cultured skin fibroblasts. There are no specific therapies for this disease.

The mucopolysaccharidoses (MPS)

MPS are lysosomal storage diseases caused by mutations in enzymes involved in the metabolism of glycosaminoglycans or mucopolysaccharides. Consequently, dermatan sulfate, heparan sulfate, keratan sulfate or chondroitin sulfate can accumulate, leading to cell dysfunction (Scriver et al., 2001). Table 43-1 shows known MPS, which can be subdivided into four clinical categories. Hurler syndrome presents with coarse facial features, hepatosplenomegaly, corneal clouding, skeletal abnormalities and psychomotor retardation. Scheie syndrome is a milder variant, with possible survival to adulthood and normal intellect. Hunter syndrome resembles Hurler, except that the cornea is not involved. In Sanfilippo syndrome, facial coarsening, hepatosplenomegaly and skeletal abnormalities are less marked, but psychomotor retardation and behavioral disturbances are severe. Conversely, Maroteaux–Lamy syndrome patients have severe somatic manifestations but normal intellect. Morquio syndrome is associated with severe skeletal abnormalities, which have a different pattern from Hurler syndrome and intact intellect. Gangliosides GM2 and GM3 accumulate in the nervous system of MPS I, II (severe variant), MPS IIIa, IIIb and IIId, but not in MPS IS. The reason for this accumulation is unknown because enzymes affected in MPS are not involved in ganglioside metabolism. Interestingly, neurological involvement in MPS correlates with accumulation of gangliosides. Enzyme replacement and hematogenous cell transplantation are promising therapies for some MPS, especially those with minimal or no neurological involvement.

Neuronal ceroid lipofuscinoses (NCLs)

NCLs (CLNs) are characterized by accumulation of autofluorescent lipopigments in nervous tissues. Clinical hallmarks include blindness, seizures, cognitive and motor decline, and early death. Age of onset varies from infancy to adulthood. Infantile, late infantile, juvenile (also referred as Batten disease) and adult variants have been described (Goebel, 1995; Haltia, 2006). All patients except those with the adult form develop visual degeneration. Dementia and motor dysfunction are present in all variants. Infantile NCL is caused by mutations in the lysosomal acid hydrolase palmitoyl protein thioesterase 1 (CLN1), PPT1, whereas late infantile NCL2 is caused by mutations in lysosomal protease peptidase. Batten disease develops from mutations in battenin, a lysosomal membrane protein. Currently, NCLs remain untreatable.

PEROXISOMAL DISEASES

Peroxisomes are specialized organelles for metabolism of oxygen peroxide and of various lipids

Peroxisomes were described as microbodies by Rhodin in the early 1950s (Rhodin, 1954) and characterized biochemically by de Duve (De Duve & Baudhuin, 1966). They are single-membrane cell organelles that range from 0.1–1 μm in diameter. Although they are typically spherical in shape, there is increased evidence of tubular or even reticular shaped peroxisomal structures (Espeel et al., 1997; Fahimi & Baumgart, 1999). This organelle is present in all cell types and tissues in numbers ranging from hundreds to thousands (Chang et al., 1999). The name peroxisome refers to their function in producing hydrogen peroxide, which is degraded by peroxisomal

catalase. Peroxisomes metabolize various lipids, most importantly through α-oxidation, β-oxidation and ether lipid synthesis (Wanders & Tager, 1998; Van Veldhoven, 2010).

Peroxisomal dysfunction and the nervous system: peroxisomal defects impair the function of systemic organs and of the nervous system

These abnormalities include white matter disease and neuronal involvement, such as defective neuronal migration (Aubourg, 2007). These features underline the relevance of these organelles in the nervous system. Neurons and oligodendrocytes rely on appropriate control of lipid metabolism. Consequently, peroxisomal dysfunction will have a detrimental impact in these cells. For example, defects in peroxisomal β-oxidation of lipids favors accumulation of very-long-chain fatty acids (VLCFA), bile acid and other lipid intermediates, promoting autoimmune responses with perivascular infiltration of T- and B-cells and macrophages as well as destabilization of myelin membranes and demyelination (Moser & Moser, 1996; Faust et al., 2010). White matter abnormalities can also be triggered by a low synthesis of plasmalogens, which are important constituents of myelin and which are produced in peroxisomes (Brites et al., 2009).

Peroxisomes appear to play an important role on the migration of specific neuronal cells (Janssen et al., 2003). Interestingly, neither accumulation of VLCFA nor lack of plasmalogens seems to be involved in neuronal migration (Powers, 1995; Baes et al., 2002). Mechanism(s) mediating regulation of neuronal migration by peroxisomes remain largely unknown. It may involve nonperoxisomal genes or even environmental factors (Gressens, 2006). Involvement of the nervous system in peroxisomal disease may also depend on the relation between peroxisomal and mitochondrial functions. In turn, given the high energy demands of neurons, altered mitochondrial function may contribute substantially to neurological defects in peroxisomal diseases (Oezen et al., 2005).

CLASSIFICATION OF PEROXISOMAL DISEASES

Human diseases involving peroxisomal dysfunction were originally described as syndromes

Over the years, reports of peroxisomal dysfunction and a better understanding of peroxisomal function helped relate genotype and phenotype in these diseases (Baes et al., 2009). Human peroxisomal diseases are classified into defects of peroxisomal biogenesis and defects of a peroxisomal protein (Moser et al., 1991; Naidu & Moser, 1990) (Tables 43-2 & 43-3).

Defects of peroxisomal biogenesis

The PEX genes encode peroxin proteins, which comprise matrix proteins, cytosolic receptors and peroxisomal membrane proteins (Steinberg et al., 2006). They are synthesized on free cytosolic ribosomes and targeted to peroxisomes by C- or N-terminal consensus sequences known as peroxisomal target sequences (PTS). Fifteen human PEX genes are known. If the defect compromises formation or import of peroxisomal membrane proteins, the phenotype is severe because organelles are not formed. In other cases, defects involving formation of matrix proteins generate membranous structures that resemble peroxisomes but are not functional (peroxisomal ghosts).

Diseases of peroxisomal biogenesis are inherited in an autosomal-recessive manner. These entities differ in clinical severity but share some features (Scriver et al., 2001; Shimozawa, 2007) and are subclassified into the Zellwenger syndrome (ZS) spectrum and the Rhizomelic condrodysplasia punctata (RCDP) spectrum (Table 43-2). The ZS spectrum group comprises three different entities: Zellwenger syndrome (ZS), neonatal adrenoleukodystrophy (NALD), and infantile Refsum disease (IRD). These entities differ in accumulation of VLCFA, phytanic acid and bile acid intermediates. The most severe form is Zellwenger syndrome. Infants with ZS live only a few months and show liver involvement, hypotonia, seizures, feeding difficulties and apnea. RCDP is characterized by deficient synthesis of plasmalogens but normal levels of VLCFA. Patients with RCDP live one or two years and show dysmorphism with disturbed endochondrial bone formation and severe psychomotor delay.

Defects of single peroxisomal enzymes

Diseases under this classification include mutations affecting (1) β-oxidation (acyl-CoA oxidase, D-bifunctional protein, peroxisomal thiolase and 2-methylacyl-CoA racemase); (2) isoprenoid biosynthesis (mevalonate kinase); (3) etherphospholipid synthesis (dihydroxyacetonephosphate acyltransferase and alkyldihydroxyacetonephosphate synthase; (4) glyoxylate detoxification; (5) phytanic acid α-oxidation; (6) L-pipecolate degradation; (7) glutaryl-CoA metabolism; and (8) hydrogen peroxide metabolism (Scriver et al., 2001; Wanders & Waterham, 2006).

These diseases are inherited as autosomal recessive traits except for X-linked adrenoleukodystrophy (X-ALD). (Examples of some single peroxisomal enzyme defects are shown in Table 43-3.) X-ALD is the most common single peroxisomal disease with an incidence of 1:20.000–1:17.000 males. About 50% of heterozygous ALD women develop a syndrome in middle age due to random inactivation of the X chromosome. X-ALD comprises various forms characterized by defective ABCD1 gene (Wanders & Waterham, 2006). This gene encodes a peroxisomal membrane protein responsible for mobilizing VLCFA into the peroxisomes for degradation. Dysfunction of this gene causes accumulation of VLCFA, primarily in white matter and adrenocortical cells. Because VLCFAs are normal components of other lipids, deficiencies in peroxisomal degradation of VLCFA also affect metabolism of lipids such as sphingomyelin, gangliosides and glycerophosphatides. Affected males can present three major phenotypes: (1) a cerebral form, (2) adrenomyeloneuropathy (AMN), and (3) Addison phenotype. Cerebral forms can present in childhood, adolescence or adulthood, in order of decreasing severity. Initial manifestations include attention deficit and behavioral disturbances with progression to dementia. This form of X-ALD is associated with inflammatory myelinopathy. The AMN form is the most common X-ALD variant,

TABLE 43-2 Diseases of Peroxisomal Biogenesis

Name	Disease forms	Biochemistry	Genetics and defective genes
Zellweger syndrome spectrum	Zellweger syndrome (ZS)	Accumulation of VLCFA, phytanic acid and bile intermediates and plasmalogens biosynthesis	Autosomal recessive PEX1, PEX2, PEX3, PEX5, PEX6, PEX10, PEX12, PEX 13, PEX16.
	Neonatal adrenoleukodystrophy (NALD)	Deficiency of plasmalogens, high phytanic acid and normal VLCFA	
	Infantile Refsum disease (IRD)		
Rhizomelic chondrodysplasia punctata (RCDP)		Deficiency of plasmalogens, high phytanic acid and normal VLCFA	Autosomal recessive PEX7

TABLE 43-3 Single Peroxisomal Enzyme Diseases

Name	Disease forms	Biochemistry	Genetics and defective genes
Pseudoneonatal adrenoleukodystrophy		elevated VLCFA	Autosomal recessive Acyl-CoA oxidase
Peroxisomal bifunctional enzyme deficiency		elevated VLCFA, bile acid intermediates	Autosomal recessive Bifunctional protein
Pseudo-Zellweger syndrome		elevated VLCFA, and bile acid intermediates	Autosomal recessive Thiolase
X-linked adrenoleukodystrophy (X-ALD)	Cerebral ALD Adrenomyeloneuropathy (AMN) Addison disease	elevated VLCFA	X-linked ALD gene
Refsum disease		elevated phytanic acid	Autosomal recessive Phytanoyl CoA hydroxylase (PAHX)

present in 45% of male patients. AMN starts in the third decade of life with paraparesis, and continues with inflammatory responses in brain, concomitant with disease progression. X-ALD patients with Addison phenotype present with adrenocortical insufficiency, initially without neurological problems, which develop later, as the disease progresses. Other single peroxisomal disorders have been reported (Scriver et al., 2001; Platt & Walkley, 2004).

THERAPY OF PEROXISOMAL DISEASES

There is currently no treatment for peroxisomal diseases caused by defects in organellar biogenesis due to the severity of systemic developmental defects. The therapeutic success in peroxisomal enzyme defects (cerebral X-ALD forms) depends on how early the mutation is identified, thus allowing immediate intervention with preventive diets and/or bone marrow transplantation from HLA-compatible donors (Aubourg et al., 1990). These are the only treatments with beneficial effects on progression of peroxisomal diseases, capable of stabilizing or even reversing demyelination. The beneficial effect of bone marrow transplantation involves secretion of the missing enzyme by donor cells and/or immunosuppression. A diet poor in VLCFA and rich in erucic acid and oleic acid (known as "Lorenzo's oil") leads to normalization of VLCFA plasma levels. Adrenal steroid replacement therapy is necessary for all patients with adrenocortical dysfunction.

DISEASES OF CARBOHYDRATE AND FATTY ACID METABOLISM

Defects of energy metabolism cause profound disturbances in muscle and brain function. Such defects may present as a myopathy, encephalopathy or encephalomyopathy. Clinical features are best appreciated by understanding the preferred oxidizable substrates for brain and muscle.

Muscle in the resting state predominantly utilizes fatty acids. The immediate source of energy for muscle contraction is ATP, which is rapidly replenished at the expense of creatine phosphate by creatine kinase phosphorylation of ADP. During exercise of moderate intensity, the fuel choice depends on the duration of work. Initially, glycogen is the main fuel source;

after 5 or 10 min, blood glucose becomes the more important fuel. As work continues, fatty acid utilization increases, and after approximately 4 hr, lipids are the primary source of energy. During high-intensity exercise at near-maximal power, additional ATP is generated by anaerobic breakdown of glycogen and glycolysis. Intense exercise is performed in essentially anaerobic conditions, whereas mild or moderate exercise is accompanied by increased blood flow to exercising muscles, facilitating substrate delivery and favoring aerobic metabolism.

Brain utilizes glucose predominantly, with regional variations of metabolic rate depending on the mental or motor task being performed. As with muscle, the immediate intracellular energy source is ATP, buttressed by creatine phosphate stores. Glycogen provides very little energy reserve because brain concentrations of glycogen are extremely low, approximately only one-tenth the amount in muscle per gram wet weight. Therefore, brain is exquisitely sensitive to fluctuations in blood glucose concentration. Movement of glucose across the blood–brain barrier is facilitated by a carrier protein, the glucose transporter (GLUT-1) (De Vivo et al., 1991). Facilitated transport of glucose ensures adequate brain glucose concentrations to meet the needs of cerebral metabolism under normal conditions. Interestingly, most brain glycogen is present in astrocytes, where it is metabolized to lactate, which is transferred to adjacent neurons and used aerobically as fuel (Brown et al., 2007). During starvation, the brain uses little, if any, fatty acids. However, fatty acids of varying chain lengths may be taken up by brain, as efficiency of transport across the blood–brain barrier is much greater for short- or medium-chain fatty acids than long-chain fatty acids. Ketone bodies represent the preferred cerebral fuel source during starvation when glucose supply is limited (DiDonato & Taroni, 2008) (see Ch. 11). Defective fatty acid oxidation, therefore, may affect muscle directly by blocking oxidation of this substrate and brain indirectly by limiting hepatic ketogenesis. Elevated circulating free fatty acids may also have a direct toxic effect on brain, but the precise mechanisms for this effect are poorly understood.

Energy metabolism has been studied extensively in skeletal muscle, and several metabolic disorders have been documented (DiDonato & Taroni, 2008; DiMauro & Bonilla, 2004). Comparatively less is known about metabolic defects in cerebral energy metabolism. This may be because muscle tissue is more accessible for biochemical analysis and certain cerebral enzyme defects are lethal.

Diseases of carbohydrate and fatty acid metabolism in muscle

One class of glycogen or lipid metabolic disorders in muscle is manifest as acute, recurrent, reversible dysfunction

These disorders occur with exercise intolerance and myoglobinuria, with or without cramps. Among the glycogenoses, this is characteristic of deficiencies in phosphorylase, phosphofructokinase (PFK), aldolase, phosphoglycerate kinase (PGK), phosphoglycerate mutase (PGAM), β-enolase and lactate dehydrogenase (LDH). Among disorders of lipid metabolism, this is characteristic of deficiencies in very-long-chain acyl-CoA dehydrogenase (VLCAD), trifunctional protein (TP), carnitine palmitoyltransferase II (CPT II) and short-chain 3-hydroxyacyl-CoA dehydrogenase (Janssen et al., 2003). Figures 43-2 and 43-3 schematically illustrate pathways of glycogen and fatty acid metabolism.

Phosphorylase deficiency (McArdle disease, glycogenosis type V) exemplifies the glycogenoses causing recurrent muscle "energy crises," with cramps, myalgia, and, often, rhabdomyolysis and myoglobinuria

This is an autosomal recessive myopathy caused by mutations in the muscle isoenzyme of glycogen phosphorylase (Fig. 43-2). Intolerance of strenuous exercise is present from childhood, but usually onset is in adolescence, with cramps after exercise (DiMauro & Bonilla 2004). Myoglobinuria occurs in about half of patients. If they avoid intense exercise, most patients can live normal lives; however, about one-third develops some degree of fixed weakness, usually as a late-onset manifestation of the disease. In a few patients, weakness rather than exercise-related cramps and myoglobinuria, characterizes the clinical picture.

In patients with myoglobinuria, renal insufficiency is a possible life-threatening complication. Physical examination between episodes of myoglobinuria may be completely normal or show some degree of weakness and, occasionally, wasting of some muscle groups. Even between episodes, most patients have increased serum creatine kinase (CK); forearm ischemic exercise causes no rise of venous lactate concentration. This is a useful but nonspecific test in McArdle disease. The electromyogram (EMG) at rest shows nonspecific myopathic features in about half of patients.

Muscle biopsy demonstrates subsarcolemmal blebs that contain periodic-acid-Schiff (PAS)–positive material, a marker for glycogen (DiMauro & Bonilla, 2004). The histochemical stain for phosphorylase is negative, except in regenerating fibers. Biochemical documentation of enzyme deficiency requires muscle biopsy because the defect is not expressed in more easily accessible tissues, such as leukocytes, erythrocytes and cultured fibroblasts. The gene encoding muscle phosphorylase (PYGM) is assigned to chromosome 11, and more than 60 distinct mutations have been identified in patients (Lucia et al., 2008). By far the most common is a nonsense mutation in codon 50 (mut-50) allowing diagnosis through molecular analysis of genomic DNA from blood, thus making muscle biopsy unnecessary (Lucia et al., 2008).

Genetic defects of phosphorylase b kinase (PHK)

Not too surprisingly, genetic defects of phosphorylase b kinase (PHK), the main activating enzyme of phosphorylase, cause a less severe form of McArdle syndrome, which is X-linked because mutations affect the α_M subunit, a muscle-specific protein encoded by a gene (PHKA1) on the X-chromosome.

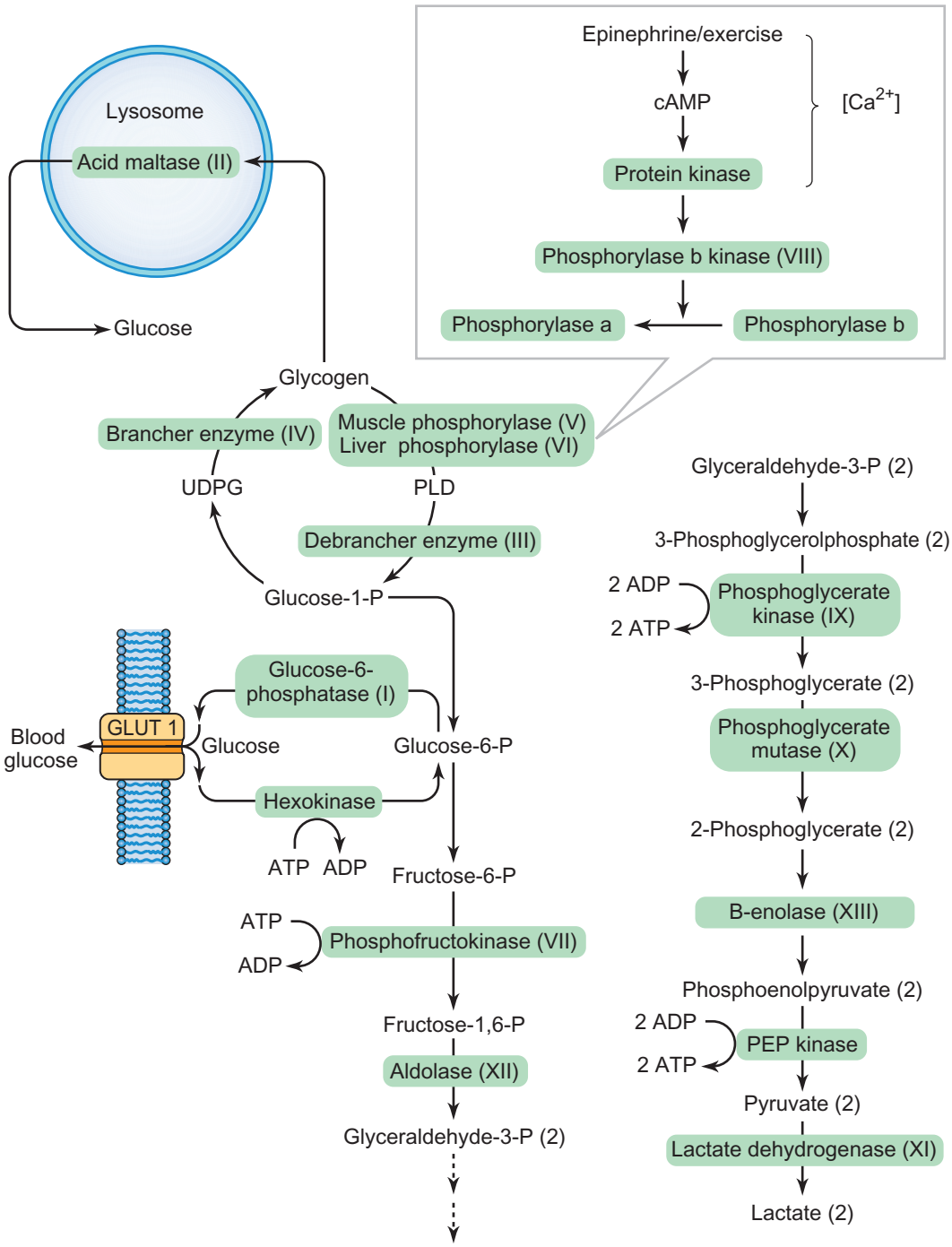

FIGURE 43-2 **Schematic representation of glycogen metabolism and glycolysis**. *Roman numerals* indicate the sites of identified enzyme defects: *I*, glucose-6-phosphatase; *II*, acid maltase; *III*, debrancher enzyme; *IV*, brancher enzyme; *V*, muscle phosphorylase; *VI*, liver phosphorylase; *VII*, phosphofructokinase; *VIII*, phosphorylase kinase; *IX*, phosphoglycerate kinase; *X*, phosphoglycerate mutase; *XI*, lactate dehydrogenase; *XII*, aldolase; *XIII*, β-enolase. *GLUT 1*, glucose transporter; *P*, phosphate; *PEP*, phosphoenolpyruvate; *PLD*, phosphorylase-limit dextrin; *UDPG*, uridine diphosphate glucose.

Other glycolytic defects involving PFK, PGK, PGAM, and LDH have clinical and pathological features similar to McArdle disease

Patients with PFK deficiency (glycogenosis type VII, Tarui disease) may have mild jaundice, reflecting excessive hemolysis. This is because the muscle-specific subunit of this tetrameric enzyme is also present in erythrocytes, which have partial PFK deficiency. A unique pathological feature of PFK deficiency is the presence in muscle of a small portion of the abnormal glycogen, which,. by histochemical

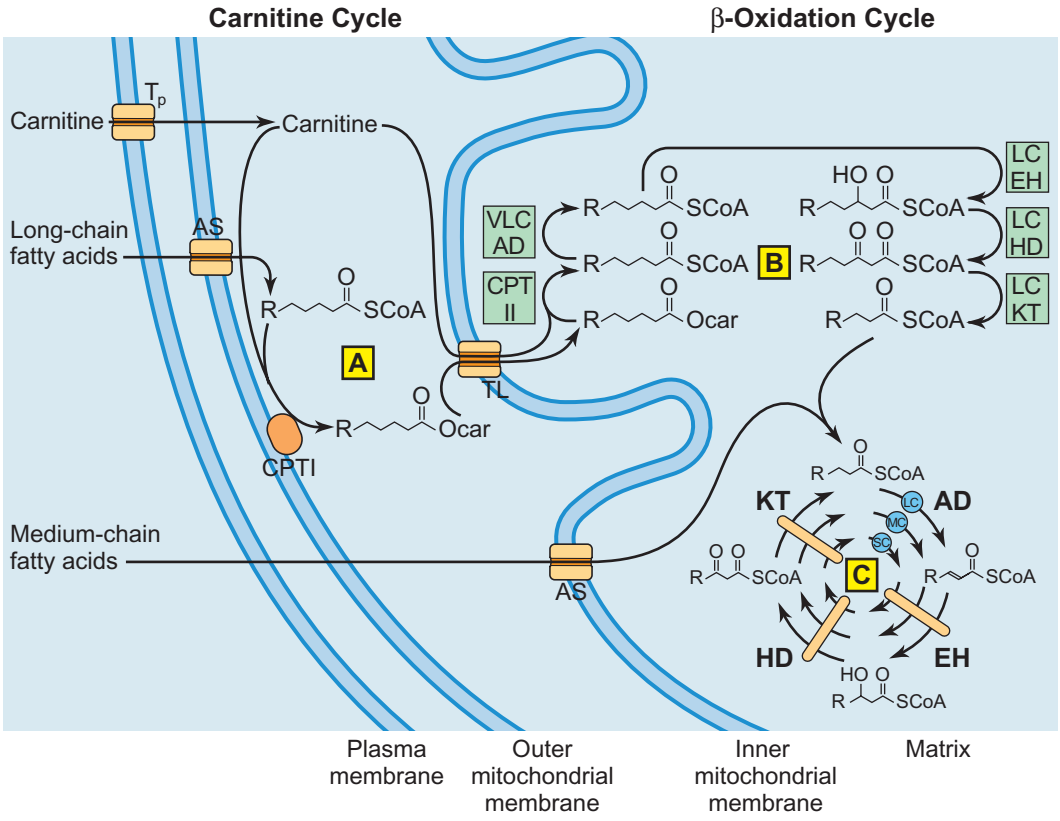

FIGURE 43-3 **Schematic representation of fatty acid oxidation**. This metabolic pathway is divided into the carnitine cycle **(A)**, the inner mitochondrial membrane system **(B)**, and the mitochondrial matrix system **(C)**. The carnitine cycle includes the plasma membrane transporter (*Tp*), carnitine palmitoyltransferase I (*CPT I*), the carnitine–acylcarnitine translocase system (*Tl*) and carnitine palmitoyltransferase II (*CPT II*). The inner mitochondrial membrane system includes the very-long-chain acyl-CoA dehydrogenase (*VLCAD*) and the trifunctional protein with three catalytically active sites. Long-chain acylcarnitines enter the mitochondrial matrix by the action of CPT II to yield long-chain acyl-CoAs. These thioesters undergo one or more cycles of chain shortening catalyzed by the membrane-bound system. Chain-shortened acyl-CoAs are degraded further by the matrix β-oxidation system. Medium-chain fatty acids enter the mitochondrial matrix directly and are activated to the medium-chain acyl-CoAs before degradation by the matrix β-oxidation system. *AD*, acyl-CoA dehydrogenase; *AS*, acyl-CoA synthetase; *CoA*, coenzyme A; *CPT*, carnitine palmitoyltransferase; *EH*, 2-enoyl-CoA hydratase; *HD*, 3-hydroxyacyl-CoA dehydrogenase; *KT*, 3-ketoacyl-CoA thiolase; *LC*, long chain; *MC*, medium chain; *SC*, short chain; *TL*, carnitine-acylcarnitine translocase; T_p, carnitine transporter; *VLC*, very long chain. (With permission from reference Platt & Walkley, 2004.)

analysis, is diastase-resistant, and by electron microscopy, has a finely granular and filamentous structure, similar to the poorly branched polyglucosan that accumulates in branching enzyme deficiency. The presence of polyglucosan is probably due to the increased concentration of glucose 6 phosphate (G6P) resulting from the enzyme defect. As G6P is a physiological activator of glycogen synthetase, its excess may keep an abnormally high proportion of glycogen synthetase in the active form, thus altering the delicate balance between synthetase and branching enzyme activities in favor of glycogen chain elongation. Direct evidence for the validity of this mechanism comes from the discovery that polyglucosan myopathy in horses is due to a gain-of-function mutation in glycogen synthetase (McCue et al., 2008).

Patients with phosphoglycerate kinase (PGK) deficiency, an X-linked recessive disease (type IX, Fig. 43-2) show involvement of three tissues in various combinations, erythrocytes, skeletal muscle and the CNS (Spiegel et al., 2009). The isolated myopathic presentation is characterized by exercise-induced cramps and myoglobinuria. CNS involvement in the absence of hemolytic anemia or myopathy may be underestimated and manifest as seizures, mental retardation, or juvenile parkinsonism.

About 15 patients with phosphoglycerate mutase (PGAM) deficiency have been identified. This autosomal recessive myopathy is caused by a genetic defect of the muscle subunit of PGAM (type X, Fig. 43-2). A unique pathologic feature of PGAM deficiency is the frequent presence in muscle of tubular aggregates, stacks of ordered tubular structures apparently derived from the sarcoplasmic reticulum.

Lactate dehydrogenase (LDH) deficiency is an autosomal recessive myopathy caused by a genetic defect of the muscle subunit, which is encoded by a gene on chromosome 11 (type XI, Fig. 43-2). Thus far, several Japanese families and two

Caucasian patients with this disease have been described. The clinical picture is characterized by cramps and myoglobinuria after intense exercise. Forearm ischemic exercise showed a subnormal rise of lactate concentration, contrasting with an increased rise of pyruvate.

Beta-enolase deficiency (type XIII, Fig. 43-2) has been identified in a 47-year-old man with late-onset but rapidly progressive exercise intolerance and exercise-triggered myalgia. The patient was a compound heterozygote for two mutations in the ENO3 gene, which is located on chromosome 17 (DiMauro & Bonilla, 2004).

CPT II deficiency has clinical features similar to McArdle disease

Carnitine palmitoyltransferase II (CPT II) deficiency will be used as the prototype of lipid disorders causing recurrent episodes of cramps, myalgia, and myoglobinuria. This is an autosomal recessive myopathy caused by a genetic defect of the mitochondrial enzyme CPT II (Fig. 43-3). The disease is prevalent in men (male:female ratio, 5.5:1) and appears to be the most common cause of recurrent myoglobinuria in adults (DiDonato & Taroni, 2008).

Clinical manifestations are limited to attacks of myoglobinuria, not preceded by contractures and usually precipitated by prolonged exercise, of several hours' duration; prolonged fasting; or a combination of the two conditions. Less common precipitating factors include intercurrent infection, emotional stress and cold exposure, but some episodes of myoglobinuria occur without any apparent cause. Most patients have two or more attacks, probably because the lack of muscle cramps deprives them of a warning signal of impending myoglobinuria.

For unknown reasons, some women seem to have milder symptoms, such as myalgia, after prolonged exercise, without pigmenturia. This has been observed in sisters of men with recurrent myoglobinuria. The only serious complication is renal failure following myoglobinuria.

Physical and neurological examinations are completely normal. Prolonged fasting at rest, which should be conducted under close medical observation, causes a sharp rise of serum CK in about one-half of patients. Also, in about one-half of patients, ketone bodies fail to increase normally after prolonged fasting. Forearm ischemic exercise causes a normal increase of venous lactate concentration. Aside from episodes of myoglobinuria, the serum CK concentration and EMG are normal. A muscle biopsy specimen may appear completely normal or show variable, but usually moderate, accumulation of lipid droplets. Most patients with CPT II deficiency benefit from a high-carbohydrate, low-fat diet, and the therapeutic response may serve as an indirect diagnostic clue. Because the enzyme defect appears to be generalized, tissues other than muscle, such as mixed leukocytes or isolated lymphocytes or platelets, can be used to demonstrate CPT II deficiency, but the diagnosis should be confirmed in muscle.

The gene for CPT II has been localized to chromosome 1, and several mutations have been identified in patients (DiDonato & Taroni, 2008). As in the case of McArdle disease (see above), one mutation, a serine-to-leucine substitution at codon 113, is far more common than the others and can be screened for in genomic DNA from blood cells, thus potentially avoiding muscle biopsy.

Other beta-oxidation defects have clinical features similar to McArdle disease

Very-long-chain acyl-CoA dehydrogenase (VLCAD) and trifunctional protein (Saravanan et al., 2004) are the two inner membrane-bound enzymes of fatty acid β-oxidation. Genetic defects of either can mimic the clinical presentation of CPT II deficiency by causing recurrent myoglobinuria in otherwise apparently healthy young adults. As in CPT II deficiency, precipitating factors include prolonged exercise, prolonged fasting, cold exposure, intercurrent illnesses or emotional stress (DiDonato & Taroni, 2008).

Short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency has been described in three patients. It is associated with additional defects of β-oxidation, which may cause limb weakness and attacks of myoglobinuria, and it is potentially fatal.

A second class of disorders of glucose and fatty acid metabolism causes progressive weakness

These disorders are associated with acid maltase, debrancher enzyme, and brancher enzyme deficiencies among the glycogenoses. These are also associated with carnitine deficiency, some defects of β-oxidation and other lipid-storage myopathies among the disorders of lipid metabolism. Figures 43-2 and 43-3 schematically illustrate the pathways of glycogen and fatty acid metabolism.

Acid maltase deficiency (AMD) (glycogenosis type II)

AMD is an autosomal recessive disease caused by a genetic defect of the lysosomal enzyme acid maltase, an α-1,4- and α-1,6-glucosidase capable of digesting glycogen completely to glucose (Fig. 43-2). Two major clinical syndromes are caused by AMD. The first is Pompe disease, which is a severe, generalized and invariably fatal disease of infancy; the second is a less severe neuromuscular disorder beginning in childhood or in adult life (see Ch. 41).

Infantile, generalized cardiomegalic AMD, or Pompe disease, usually becomes manifest in the first weeks or months of life, with failure to thrive, poor suck, generalized hypotonia and weakness, also termed "floppy infant syndrome." Macroglossia is common, as is hepatomegaly, which, however, is rarely severe. There is massive cardiomegaly, with congestive heart failure. Weak respiratory muscles make these infants susceptible to pulmonary infection; death usually occurs before the age of 1 year and invariably before the age of 2 years, although introduction of enzyme replacement therapy (ERT) has changed this dismal prognosis (Kishnani et al., 2007).

The childhood- and adult-onset forms of AMD cause signs and symptoms that are limited to the musculature, with progressive weakness of truncal muscles and of proximal, more than distal, limb muscles, usually sparing facial and extraocular muscles. In the childhood form, onset is in infancy or childhood and progression tends to be rapid. In the adult form, onset usually is in the third or fourth decade but occasionally even later and the course is slower.

The clinical picture in male children can closely resemble Duchenne-type muscular dystrophy; in adults, it mimics limb-girdle dystrophy or polymyositis. The early and severe involvement of respiratory muscles in most patients with

AMD is a distinctive clinical clue. Respiratory failure and pulmonary infection are the most common causes of death and the effects of ERT have been less dramatic.

Serum CK is consistently increased in all forms of AMD. Forearm ischemic exercise causes a normal rise of venous lactate concentration in patients with childhood or adult AMD. The electrocardiogram (ECG) is altered in Pompe disease, with a short P–R interval, giant QRS complexes and left ventricular or biventricular hypertrophy, but is usually normal in the later-onset forms. The EMG shows myopathic features and fibrillation potentials, bizarre high-frequency discharges and myotonic discharges.

Muscle biopsy shows vacuolar myopathy of very severe degree affecting all fibers in Pompe disease but of varying degree and distribution in childhood and adult AMD. In adult AMD, biopsy specimens from unaffected muscles may appear normal by light microscopy. The vacuoles contain PAS-positive material, a marker for glycogen. Electron microscopy shows abundant glycogen, both within membranous sacs, presumably lysosomes, and free in the cytoplasm.

The enzyme defect is expressed in all tissues, and the diagnosis can be made by biochemical analysis of urine, lymphocytes (mixed leukocytes do not give reliable results) or cultured skin fibroblasts. Fibroblasts cultured from amniotic fluid can be used for prenatal diagnosis of Pompe disease. The gene encoding acid maltase is on chromosome 17, and numerous mutations have been identified in patients with both forms of AMD, confirming that infantile and late-onset AMD are allelic disorders. Predictably, more severe mutations are associated with Pompe disease; however, many patients are compound heterozygotes, and there is no strict genotype/phenotype correlation.

Debrancher enzyme deficiency (glycogenosis type III, Cori's disease, Forbe disease)

Debrancher enzyme deficiency is an autosomal recessive disease (Fig. 43-2). In its more common presentation, debrancher enzyme deficiency causes liver dysfunction in childhood, with hepatomegaly, growth retardation, fasting hypoglycemia and seizures (DiMauro & Bonilla, 2004). Myopathy has been described in about 20 patients (DiMauro & Bonilla, 2004). In most, onset of weakness was in the third or fourth decade. Wasting of distal leg muscles and intrinsic hand muscles is common, and the association of late-onset weakness and distal wasting often suggests the diagnosis of motor neuron disease or peripheral neuropathy. The course is slowly progressive. In a smaller number of patients, onset of weakness is in childhood, with diffuse weakness and wasting. The association of hepatomegaly and growth retardation facilitates the diagnosis.

There is no glycemic response to glucagon or epinephrine (Fig. 43-2), whereas a galactose load causes a normal glycemic response. Forearm ischemic exercise produces a blunted venous lactate rise or no response. Serum CK activity is variably, often markedly, increased. The ECG shows left ventricular or biventricular hypertrophy in most patients, and the EMG may show myopathic features alone or associated with fibrillations, positive sharp waves and myotonic discharges. This "mixed" EMG pattern in patients with weakness and distal wasting often reinforces the erroneous diagnosis of motor neuron disease. Motor nerve conduction velocities are moderately decreased in one-fourth of patients, suggesting a polyneuropathy. Muscle biopsy shows severe vacuolar myopathy with glycogen storage. On electron microscopy, the vacuoles correspond to pools of glycogen free in the cytoplasm. The gene for the debrancher enzyme has been assigned to chromosome 1, and more than 15 mutations have been identified in patients (DiMauro & Bonilla, 2004).

Branching enzyme deficiency (glycogenosis type IV; Andersen's disease)

Branching enzyme deficiency is an autosomal recessive disease of infancy or early childhood, typically causing liver dysfunction with hepatosplenomegaly, progressive cirrhosis and chronic hepatic failure (Fig. 43-2). Death usually occurs in childhood. However, myopathy has been underdiagnosed. It occurs either alone or in association with hepatopathy, cardiopathy, or encephalopathy. Some infants had severe hypotonia, wasting, contractures and hyporeflexia, suggesting the diagnosis of spinal muscular atrophy (DiMauro & Bonilla, 2004).

Muscle biopsy may be normal or show focal accumulations of abnormal glycogen, which is intensely PAS-positive and partially resistant to diastase digestion (polyglucosan). With the electron microscope, the abnormal glycogen is found to have a finely granular and filamentous structure. The gene that encodes the branching enzyme has been assigned to chromosome 3 and several mutations have been identified in patients. DiMauro & Bonilla (2004).

Carnitine deficiency

Carnitine deficiency may be tissue-specific or generalized. Tissue-specific carnitine deficiency has previously been termed myopathic carnitine deficiency because patients have generalized limb weakness, starting in childhood. Limb, trunk and facial musculature may be involved. The course is slowly progressive, but weakness may fluctuate in severity. Laboratory investigations show normal or near-normal serum carnitine concentrations and variably increased serum CK values. The EMG shows myopathic features with or without spontaneous activity at rest. Muscle biopsy reveals severe triglyceride storage, best seen with the oil red O stain in frozen sections. This condition, transmitted as an autosomal recessive trait, was thought to be due to a defect of the active transport of carnitine from blood into muscle. However, no such defect has ever been documented. Rather, an increasing number of patients have a tissue-specific defect involving the short-chain isoform of acyl-CoA dehydrogenase (SCAD).

Systemic carnitine deficiency is due to a defect of the *SLC22A5* gene that encodes an integral plasma membrane protein, cation transporter 2 (OCTN2), which transports extracellular carnitine into cells. This genetically determined defect of membrane carnitine transport is the only known condition that fulfills the criteria for primary carnitine deficiency (DiDonato & Taroni, 2008; Lamhonwah et al., 2002). It is transmitted as an autosomal recessive trait and produces a life-threatening cardiomyopathy in infancy or early childhood, which is effectively treated with carnitine supplementation. The untreated patient also manifests systemic features of hypotonia, failure to thrive and alterations of consciousness, including coma. Carnitine concentrations are extremely low in

plasma and body tissues, and the excretion of carnitine in the urine is extremely high. The excessive urinary carnitine losses are caused by a defect in renal tubular uptake of filtered carnitine, resulting from the primary defect of the plasma membrane carnitine transporter. This condition can be documented by carnitine-uptake studies in cultured skin fibroblasts from patients. Uptake studies in parents give intermediate values, consistent with a heterozygous state. The defect has been documented in cultured fibroblasts and muscle cultures, but the same uptake system is probably shared by heart and kidney, thus explaining the cardiomyopathy and the excessive "leakage" of carnitine into the urine. Oral l-carnitine supplementation results in dramatic improvement in cardiac function (Lamhonwah et al., 2002).

Although systemic carnitine deficiency was first described in 1975, many of the original patients have been reinvestigated and found to have a primary enzyme defect, such as medium-chain acyl-CoA dehydrogenase deficiency (MCAD). This deficiency is the prototype of a defect in β-oxidation that produces secondary carnitine deficiency. β-Oxidation defects also are associated with dicarboxylic aciduria. This finding is particularly prominent during a metabolic crisis and may be rather inconspicuous between attacks. The differential diagnosis of systemic carnitine deficiency and dicarboxylic aciduria includes other defects of β oxidation, such as deficiencies of the long-chain isoform of acyl-CoA dehydrogenase (LCAD), SCAD, electron transfer flavoprotein (ETF) and ETF oxidoreductase (DiDonato & Taroni, 2008), the long- and short-chain isoforms of 3-hydroxyacyl CoA dehydrogenase (LCHAD, SCHAD), β-ketothiolase, and the TP enzyme that includes the catalytic activities of enoyl hydratase, LCHAD and β-ketothiolase. Cardiac involvement is particularly prominent in conditions that involve the metabolism of long-chain fatty acids. Other genetically determined biochemical defects involving organic acid metabolism and respiratory chain function may produce secondary carnitine deficiency. Carnitine deficiency also may result from acquired diseases, such as chronic renal failure treated by hemodialysis, renal Fanconi syndrome, chronic hepatic disease with cirrhosis and cachexia, kwashiorkor and total parenteral nutrition in premature infants. The mechanisms of carnitine depletion in these diverse conditions include excessive renal loss and excessive accumulation of acyl-CoA thioesters. These potentially toxic compounds are esterified to acylcarnitines and excreted in the urine, resulting in an excessive loss of carnitine.

A few patients have been described with a defect involving the carnitine—acylcarnitine translocase system, which facilitates the movement of long-chain acylcarnitine esters across the inner membrane of the mitochondrion (Fig. 43-3). These patients have extremely low carnitine concentrations and minimal dicarboxylic aciduria (DiDonato & Taroni, 2008).

Carnitine concentrations are normal to high in patients with a primary defect of CPT I. Patients with CPT II have normal carnitine concentrations. Two clinical syndromes have emerged in relationship to CPT II. The more common syndrome, as discussed previously, involves recurrent myoglobinuria provoked by fasting or intercurrent infection and later may be associated with fixed limb weakness. The less common syndrome involves infants and produces hypoketotic hypoglycemic coma with a Reye-like clinical signature. All cases thought to be recurrent Reye's syndrome should be investigated for defects involving fatty acid oxidation. Low serum carnitine concentrations and increased urinary dicarboxylic acids implicate a biochemical defect of β oxidation. Low serum carnitine concentrations and normal urinary dicarboxylic acids implicate a defect of the membrane carnitine transporter or the mitochondrial inner membrane carnitine—acylcarnitine translocase system. Normal to high serum carnitine concentrations and no dicarboxylic aciduria suggests a defect of CPT I or CPT II.

Oral administration of l-carnitine is life-saving in patients with the genetically determined defect of the plasma membrane carnitine transporter (DiDonato & Taroni, 2008; Lamhonwah et al., 2002). It also is recommended as a supplement in all patients who have documented carnitine deficiency, even though clear evidence of benefit is lacking. Medium-chain triglyceride supplementation has proven beneficial in CPT I deficiency and should be beneficial also in the other defects of the carnitine cycle. Medium-chain fatty acids cross the plasma membrane and the mitochondrial membranes directly and are esterified to the thioesters in the mitochondrial matrix (Fig. 43-3). A ketonemic response to medium-chain triglycerides documents the biological integrity of β oxidation and implicates a biochemical defect of the carnitine cycle or of β oxidation involving the metabolism of the longer-chain fatty acids.

Defects in adipose triglyceride lipase (ATGL)

Defects of ATGL impair the utilization of TG stored in lipid droplets and cause generalized neutral lipid storage disease (NLSD), of which there are two clinical variants, one characterized by ichthyosis (NLSDI or Chanarin Dorfman syndrome), the other dominated by myopathy (NLSDM). Both conditions cause adult-onset, slowly progressive weakness and massive lipid storage in muscle, best revealed histochemically with the oil-red-O, the Sudan black, or the Nile red stains. The heart can also be affected in NLSDM. The generalized nature of these disorders is manifested by the accumulation of triglyceride droplets in granulocytes, which can easily be seen in a blood smear (Jordans anomaly) and are an important diagnostic clue. NLSDM is due to mutations in the *PNPLA2* gene and NLSDI is due to mutations in the *CGI-58* gene. The lipase encoded by *PNPLA2* catalyzes the initial step of TG hydrolysis whereas the protein encoded by *CGI-58* is a co-activator of the same process (Ohkuma et al., 2009).

The impairment of energy production, be it from carbohydrate or lipids, is expected to lead to common consequences and result in similar exercise-related signs and symptoms

Except for debrancher deficiency, this is the case (DiMauro & Bonilla, 2004). Of the nine glycolytic enzyme defects described above, eight affect glycogen breakdown or glycolysis: phosphorylase, debrancher, PFK, aldolase, PGK, PGAM, β-enolase, LDH deficiencies. Patients with phosphorylase, PFK, aldolase, PGK, β-enolase, PGAM or LDH deficiency have exercise intolerance manifested by premature fatigue, cramps and, often, myoglobinuria. As predicted by the crucial role of glycogen as a fuel source, these patients are more prone to experience cramps and myoglobinuria when they engage in isometric

exercise, such as lifting weights, or in intense dynamic exercise, such as walking uphill. Energy for these types of exercise derives mainly from anaerobic or aerobic glycolysis. The block of glycogen utilization leads to a shortage of pyruvate and, therefore, of acetyl CoA (Fig. 43-4), the pivotal substrate of the Krebs cycle, and to a decreased mitochondrial energy output. In McArdle disease, even moderate exercise typically causes premature fatigue and myalgia, but these symptoms usually resolve after brief rest or slowing of pace; thereafter, patients find that they can resume or continue exercise without problems. This second-wind phenomenon, which is typical and even diagnostic of McArdle disease, seems to be due to early mobilization of fatty acids and to increased blood flow to exercising muscles.

Conversely, patients with fatty-acid oxidation defects experience myalgia and myoglobinuria after prolonged, though not necessarily high-intensity, exercise. Fasting exacerbates these complaints. Thus, myoglobinuria occurs in CPT deficiency under metabolic conditions that favor oxidation of fatty acids in normal muscle (DiDonato & Taroni, 2008). This observation suggests that impaired cellular energy production is the common cause of myoglobinuria in diverse metabolic myopathies. However, biochemical proof of energy depletion is still necessary. No abnormal decrease of ATP concentration has yet been measured in muscle of patients with McArdle disease during fatigue, which is defined as failure to maintain the required or expected force, or during ischemic exercise-induced contracture. It cannot be excluded, however, that contracture as well as necrosis may involve only a relatively small percentage of fibers. Measurements of ATP and phosphocreatine in whole muscle might fail to detect loss of high-energy phosphate compounds in selected fibers. Additionally, ATP deficiency may affect a specific subcellular compartment.

The cause of weakness is also poorly understood. Chronic impairment of energy provision is unlikely because two of the three glycogenoses causing weakness involve a glycogen-synthesizing enzyme (branching enzyme deficiency) and a lysosomal glycogenolytic enzyme (acid maltase deficiency) (see Ch. 41), neither of which is directly involved in energy production (DiMauro & Bonilla, 2004).

A more likely explanation is that weakness may be due to a net loss of muscle fibers because regeneration cannot keep pace with the rate of degeneration. With fewer functioning fibers, the muscle cannot exert full force. EMG reinforces this interpretation: motor unit potentials are of smaller amplitude and briefer duration than normal due to loss of muscle

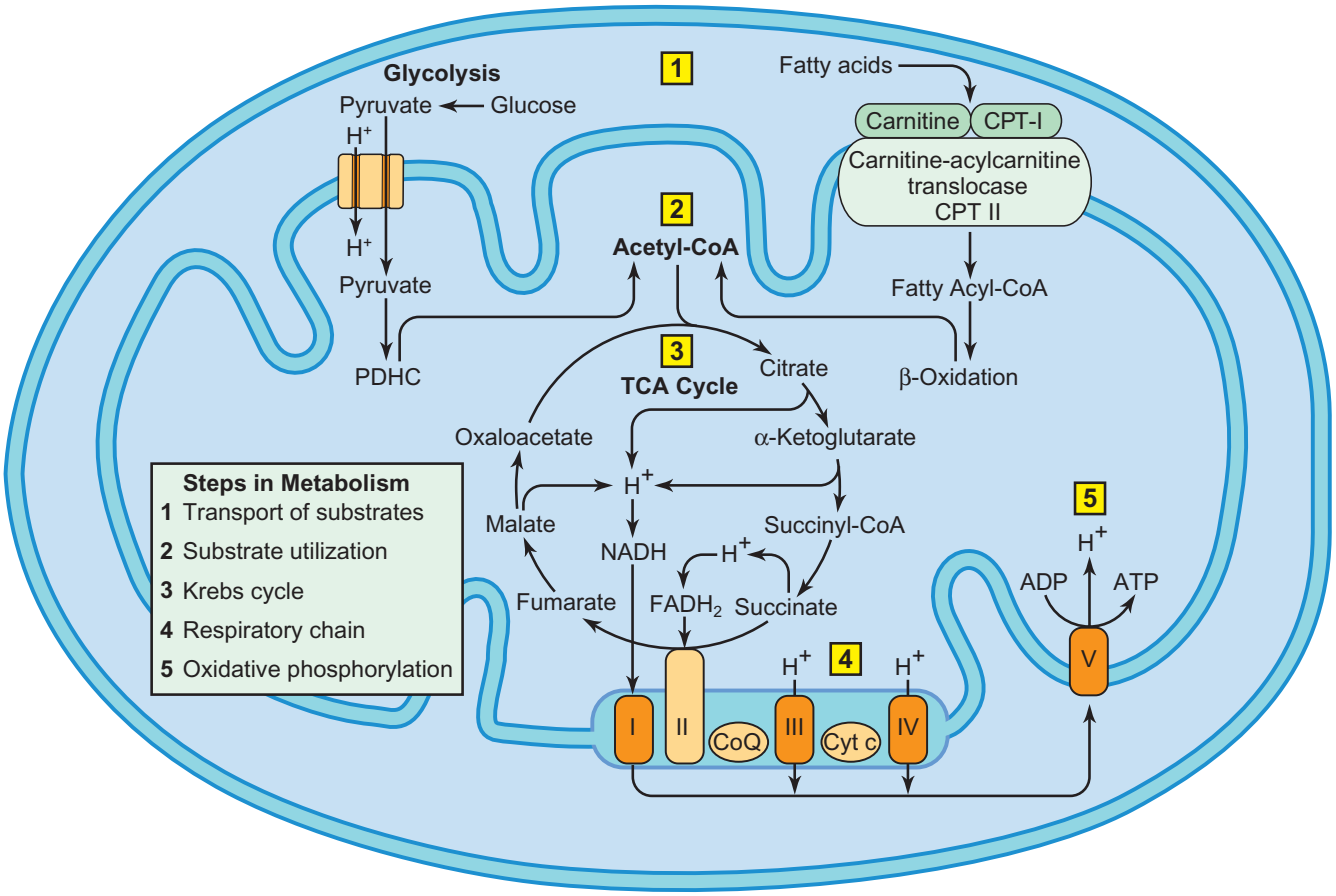

FIGURE 43-4 **Schematic representation of mitochondrial metabolism**. Respiratory chain complexes or components encoded exclusively by the nuclear genome are *light orange*. Complexes containing some subunits encoded by the nuclear genome and others encoded by mitochondrial DNA are *dark orange*. *CPT*, carnitine palmitoyltransferase; *PDHC*, pyruvate dehydrogenase complex; *CoA*, coenzyme A; *TCA*, tricarboxylic acid; *CoQ*, coenzyme Q; *Cyt c*, cytochrome c. (Modified from reference (Conradi et al., 1984), with permission of McGraw-Hill, New York).

fibers from a motor unit. Fibrillations are attributed to areas of focal necrosis of muscle fiber, isolating areas of the cell from the neuromuscular junction in a form of "microdenervation." Muscle fiber degeneration may be due to excessive storage of glycogen, as in acid maltase and debrancher enzyme deficiencies, or lipid droplets, as in carnitine deficiency or neutral lipid storage disease (NLSD). In agreement with this hypothesis is the observation that in at least two of the glycogenoses causing weakness, infantile acid maltase deficiency and debrancher enzyme deficiency, glycogen storage is much more severe than in the glycogenoses causing cramps and myoglobinuria. Similarly, lipid storage is much more severe in carnitine deficiency and in NLSD than it is in CPT deficiency (Ohkuma et al., 2009).

An additional cause of weakness may be involvement of the anterior horn cells of the spinal cord, which is very conspicuous in infantile acid maltase deficiency. All three glycogenoses causing weakness are in fact due to generalized enzyme defects, but histological signs of denervation are not evident.

Diseases of carbohydrate and fatty acid metabolism in brain

The concentration of glycogen in brain is small (approximately 0.1 g per 100 g fresh tissue, compared with 1.0 g per 100 g in muscle and 6 to 10 g per 100 g in liver) and its localization is predominantly in astrocytes (De Vivo et al., 1991). The functional significance of glycogen in the brain is not completely understood, but it is generally assumed that it represents available energy to be tapped during glucose depletion; however, the limited glycogen reserve renders the brain vulnerable to injury within minutes of onset of hypoglycemia or hypoxia.

The role of fatty acids as oxidizable fuels for brain metabolism is negligible, but ketone bodies, derived from fatty acid oxidation, can be utilized, particularly in the neonatal period. Diseases of carbohydrate and fatty acid metabolism may affect the brain directly or indirectly (DiMauro & Bonilla, 2004; De Vivo et al., 2008).

Defective transport of glucose across the blood–brain barrier is caused by deficiency in the glucose transporter protein

Glucose crosses the blood–brain barrier by a mechanism of facilitated diffusion (Chaps. 1, 3 and 11). This stereospecific system has a relatively high Km for glucose, approximately 6 mM. Normally, transport of glucose across the blood–brain barrier is not rate limiting for cerebral metabolism. Two patients were reported with a defect involving the GLUT-1 carrier protein (De Vivo et al., 2008). The clinical presentation was infantile-onset seizures and developmental delay. One patient had deceleration of head growth with resulting microcephaly. The metabolic signature of this condition is a persistent hypoglycorrhachia with low-normal or low CSF lactate values. The patients responded to a ketogenic diet that was implemented to provide ketone bodies as an alternative fuel source for cerebral metabolism (De Vivo et al., 2008). The GLUT-1 protein also is present in erythrocyte membranes. Decreased binding of cytochalasin B, a ligand that selectively binds to glucose transporters, was documented in both cases, and decreased uptake of 3-O-methylglucose by freshly isolated erythrocytes was documented in one case. The molecular and genetic basis for this condition involves mutation in the GLUT-1 gene on chromosome 1 (De Vivo et al., 2008). These patients may be misdiagnosed as examples of cerebral palsy, suspected hypoglycemia or sudden infant death syndrome. Phenotypic variants have been recognized, often dominated clinically by intermittent extrapyramidal, cerebellar or pyramidal signs triggered by environmental stress. Alternating hemiplegia of childhood, paroxysmal exercise-provoked dystonia, intermittent ataxia and hemiplegic migraine are some allelic variants of Glut1 Deficiency.

One class of carbohydrate and fatty acid metabolism disorders is caused by defects in enzymes that function in the brain

Infantile acid maltase deficiency is characterized by large amounts of glycogen in the perikaryon of glial cells in both gray and white matter, whereas cortical neurons contain much smaller quantities of glycogen. In the spinal cord, the neurons of the anterior horn appear ballooned and contain glycogen, as shown by the abundant PAS-positive material that is digested by diastase. Schwann cells of both anterior and posterior spinal roots and of peripheral nerves also contain excessive glycogen. By electron microscopy, the most striking feature is the presence of glycogen granules within membrane-bound vacuoles. These glycogen-laden vacuoles are particularly abundant in anterior horn cells, in neurons of brainstem motor nuclei and in Schwann cells, whereas they are scarce in cortical neurons. Glycogen is increased in postmortem brain, and acid maltase activity is undetectable. The severe involvement of spinal and brainstem motor neurons and the massive accumulation of glycogen in muscle contribute to the profound hypotonia, weakness and hyporeflexia seen in Pompe disease (Kishnani et al., 2007).

Debrancher enzyme deficiency

Debrancher enzyme deficiency appears to be generalized. Accordingly, although neither pathology nor debrancher enzyme activity has been reported, increased glycogen concentration has been observed in the brain of a patient. Thus, in debrancher enzyme deficiency, the nervous system seems to be involved biochemically, although clinical signs of brain dysfunction are limited to hypoglycemic seizures in childhood (DiMauro & Bonilla, 2004).

Branching enzyme deficiency

Branching enzyme deficiency has been described in multiple tissues, reflecting the fact that the enzyme is expressed as a single molecular form. Branching enzyme deficiency causes the accumulation of an abnormal glucose polymer resembling amylopectin and called polyglucosan. A form of polyglucosan body disease (adult polyglucosan body disease, APBD) occurs in patients with a characteristic neurological syndrome consisting of progressive upper and lower motor neuron involvement, sensory loss, neurogenic bladder, and, in one-half of the patients, dementia without myoclonus or epilepsy. Onset is

in the fifth or sixth decade, and the course varies between 3 and 30 years. Polyglucosan bodies are disseminated throughout the CNS in neuronal processes and astrocytes but not in perikarya. Other tissues are also affected, including peripheral nerves, liver, heart and skeletal and smooth muscle. APBD is predominant in Ashkenazi Jewish patients, in whom a "mild" mutation in the branching enzyme gene (*GBE1*) has been documented, probably due to a founder effect. The mild nature of the mutation may explain the late onset of this variant, but the preferential brain involvement remains unclear. [15292]

Phosphoglycerate kinase deficiency

Phosphoglycerate kinase deficiency in its most common manifestation, includes nonspherocytic hemolytic anemia and CNS dysfunction. Neurological problems vary in severity. All patients show some degree of mental retardation, with delayed language acquisition and behavioral abnormalities, some have hemiplegia or seizures, and three had juvenile Parkinsonism. The enzyme defect has been directly proven in the brain, and the severe brain involvement can be explained by impairment of the glycolytic pathway.

Lafora disease

Lafora disease is characterized by accumulation of polyglucosan in the CNS and PNS as well as other tissues (DiMauro & Bonilla, 2004). Lafora disease is transmitted as an autosomal recessive trait and is dominated by epilepsy, myoclonus and dementia. Other neurological manifestations include ataxia, dysarthria, spasticity and rigidity. Onset is in adolescence, and death occurs in most patients before 25 years of age.

The pathological hallmark of the disease is the presence in the brain of Lafora bodies. These are virtually identical to the polyglucosan bodies of APBD and consist of round, basophilic, PAS-positive intracellular inclusions varying in size from small, "dust-like" bodies less than 3 nm in diameter to large bodies up to 30 nm in diameter. Lafora bodies are typically seen in neuronal perikarya and dendrites and are more abundant in cerebral cortex, substantia nigra, thalamus, globus pallidus and dentate nucleus. Molecular analysis has identified mutations in two genes, *EPM2A* encoding laforin, a glycogen phosphatase, and *EPM2B* encoding malin, an E3 ubiquitin ligase. The substrate of the phosphatase activity appears to be normal glycogen, which contains a small amount of covalently linked phosphate. Mutations in laforin that impair glycogen binding also abrogate its ability to dephosphorylate glycogen, and excessive glycogen phosphorylation may lead to aberrant branching and Lafora body formation. The functional role of malin is uncertain (Ramachandran et al., 2009).

Another class of carbohydrate and fatty acid metabolism disorders is caused by systemic metabolic defects that affect the brain. Glucose-6-phosphatase deficiency (glycogenosis type I, Von Gierke disease)

Glucose-6-phosphatase deficiency results in hypoglycemia and excessive intracellular accumulation of glucose-6-phosphate (Fig. 43-2). Hypoglycemia may produce lethargy, coma, seizures and brain damage in gluconeogenic and glycogen synthetase deficiencies. As a result, there is formation of lactic acid, uric acid and lipids. A second form of the disease (type Ib) has been described. The defect in this form involves the glucose-6-phosphate translocation system that is important in facilitating the movement of the substrate into the microsomal compartment for enzymatic conversion to glucose by glucose-6-phosphatase. The clinical features of types Ia and Ib are similar, but normal enzyme activity is present in type Ib. Hepatomegaly, bleeding diathesis and neutropenia are present. The neurological signs result from the chronic hypoglycemia. Recent studies indicate that lactate may be used by the brain as an alternative cerebral metabolic fuel when hypoglycemia is associated with lactic acidosis. Nocturnal intragastric feeding and frequent daytime meals ameliorate most of the clinical and metabolic abnormalities of this condition.

Fructose-1,6-bisphosphatase deficiency

Fructose-1,6-bisphosphatase deficiency first described by Baker and Winegrad in 1970, has now been reported in approximately 30 cases. It is more common in women and is inherited as an autosomal recessive disorder. Initial manifestations are not strikingly dissimilar from those of glucose-6-phosphatase deficiency. Neonatal hypoglycemia is a common presenting feature, associated with profound metabolic acidosis, irritability or coma, apneic spells, dyspnea, tachycardia, hypotonia and moderate hepatomegaly. Lactate, alanine, uric acid and ketone bodies are elevated in the blood and urine (De Vivo et al., 2008). The enzyme is deficient in liver, kidney, jejunum and leukocytes. Muscle fructose-1,6-bisphosphatase activity is normal.

Fructose-1,6-bisphosphatase is an important rate-limiting step in gluconeogenesis. This gluconeogenic step antagonizes the opposite reaction that forms fructose-1,6-bisphosphate from fructose-6-phosphate and ATP (see Ch. 11). A futile cycle exists between these two enzymes, one forming fructose-1,6-bisphosphate and the other disposing of this substrate. Small amounts of fructose-2,6-bisphosphate also are formed by the PFK reaction. This metabolite stimulates the PFK reaction and inhibits the fructose-1,6-bisphosphatase reaction. This finding nicely explains the subtle interplay between the key rate-limiting step in glycolysis, which is PFK-dependent, and the rate-limiting step in gluconeogenesis catalyzed by fructose-1,6-bisphosphatase.

Phosphoenolpyruvate carboxykinase (PEPCK) deficiency

PEPCK is distinctly rare and even more devastating clinically than deficiencies of glucose-6-phosphatase or fructose-1,6-bisphosphatase. PEPCK activity is almost equally distributed between a cytosolic form and a mitochondrial form. These two forms have similar molecular weights but differ by their kinetic and immunochemical properties. The cytosolic activity is responsive to fasting and various hormonal stimuli. Hypoglycemia is severe and intractable in the absence of PEPCK. A young child with cytosolic PEPCK deficiency had severe cerebral atrophy, optic atrophy and fatty infiltration of liver and kidney.

Pyruvate carboxylase deficiency

Pyruvate carboxylase deficiency has been documented in 36 cases (Haymond & Sunehag, 2003). This enzyme is

mitochondrial in location and catalyzes the conversion of pyruvate to oxaloacetate in a biotin-dependent manner (Chaps. 37 and 41). The first report of pyruvate carboxylase deficiency involved an infant with subacute necrotizing encephalomyelopathy, or Leigh syndrome. Subsequent reports have failed to confirm this causal relationship between pyruvate carboxylase deficiency and the neuropathological features of Leigh syndrome. Leigh syndrome has now been assigned to several other biochemical defects, including pyruvate dehydrogenase deficiency, cytochrome–oxidase deficiency, biotinidase deficiency and defects involving complex I and complex V of the respiratory chain.

Most patients with pyruvate–carboxylase deficiency present with failure to thrive, developmental delay, recurrent seizures and metabolic acidosis. Lactate, pyruvate, alanine, β-hydroxybutyrate and acetoacetate concentrations are elevated in blood and urine. Hypoglycemia is not a consistent finding despite the fact that pyruvate carboxylase is the first rate-limiting step in gluconeogenesis.

Sixteen patients had an associated hyperammonemia, citrullinemia and hyperlysinemia. This presentation is the most malignant, with death in early infancy. This French phenotype is commonly associated with the absence of any immunological cross-reacting material (CRM) corresponding to the pyruvate carboxylase apoenzyme protein.

The North American phenotype is associated with the presence of CRM. Possibly as a result, the clinical presentation is less devastating in early infancy, although the outcome is almost invariably fatal in later infancy or early childhood. These patients do not have the associated abnormalities of ammonia metabolism, and the serum aspartic acid concentrations are not as severely depleted. Phenotype–genotype correlation is confounded by consanguinity and mosaicism, (Wang et al., 2008). Several patients have been described with the North American phenotype and a benign clinical syndrome. The first benign case had recurrent episodes of metabolic acidosis requiring hospitalization. Otherwise, her growth and neurological development have been normal.

Prenatal and postnatal diagnoses can be made by enzyme assay of cultured amniocytes, fibroblasts or white blood cells. Treatment remains symptomatic. Sodium bicarbonate is necessary to correct the acidosis. Aspartic acid supplementation will improve the systemic condition but has no effect on the neurological disturbances. Biotin supplementation is of no value.

Biotin-dependent syndromes

Biotin-dependent syndromes are manifest in infants, who may present with developmental delay and may demonstrate laboratory abnormalities resulting from deficiencies of the four biotin-dependent carboxylases (see Ch. 41). Three of the carboxylases, located in the mitochondria, are involved in organic acid metabolism. Multiple carboxylase deficiency, when present in the newborn period, is the result of a deficiency of holocarboxylase synthetase, the enzyme that catalyzes the binding of biotin to the apocarboxylase. These infants often die shortly after birth. Older infants gradually develop neurological signs, with developmental delay and seizures associated with alopecia, rash and immunodeficiency. There is a deficiency of biotinidase, the enzyme responsible for the breakdown of biocytin, the lysyl derivative of biotin, to free biotin. Biotinidase deficiency can be recognized at birth by measuring the serum activity. Biotinidase deficiency occurs in 1 in 41,000 live births, and it is eminently treatable by the oral administration of biotin.

Glycogen synthetase deficiency

Glycogen synthetase deficiency has been described in three families. It caused stunted growth and severe fasting hypoglycemia with ketonuria. Mental retardation was reported in the three children who survived past infancy. The liver was virtually devoid of glycogen and showed fatty degeneration in all cases. In two patients, the brain showed diffuse, nonspecific changes in the white matter, seen as the presence of reactive astrocytes and increased microglia, which were considered secondary to prolonged hypoglycemia or anoxia. Biochemical studies showed that glycogen-synthetase activity was markedly decreased in liver but normal in muscle, erythrocytes and leukocytes, suggesting the existence of multiple tissue-specific isoenzymes under separate genetic control.

In liver phosphorylase deficiency (glycogenosis type VI, Hers' disease; Fig. 43-2) and in two genetic forms of phosphorylase kinase deficiency, one of which is X-linked recessive, the other of which is autosomal recessive, hypoglycemia is either absent or mild. Symptoms of brain dysfunction do not usually occur (type VIII, Fig. 43-2) (DiMauro & Bonilla, 2004).

Fatty acid oxidation defects

Fatty acid oxidation defects often produce recurrent disturbances of brain function (DiDonato & Taroni, 2008; Ohkuma et al., 2009). Drowsiness, stupor and coma occur during acute metabolic crises and mimic the Reye's syndrome phenotype. The neurological symptoms have been attributed to hypoglycemia, hypoketonemia and the deleterious effects of potentially toxic organic acids. Hypoglycemia is caused by a continuing demand for glucose by brain and other organs, resulting from the primary biochemical defect of fatty-acid oxidation (Fig. 43-3). Avoidance of catabolic circumstances that require the utilization of fatty acids is the basic principle of treatment. L-carnitine supplementation is recommended for all conditions associated with generalized carnitine deficiency. Some patients may benefit from medium-chain triglyceride supplementation, as discussed previously. Certain forms of ETF-oxidoreductase deficiency respond to riboflavin supplementation. The riboflavin-responsive multiple acyl CoA dehydrogenase deficiency represents the milder form of glutaric aciduria type II.

DISEASES OF MITOCHONDRIAL METABOLISM

Mitochondrial dysfunction produces syndromes involving muscle and the central nervous system

Although some energy can be obtained quickly from glucose or glycogen through anaerobic glycolysis, most of the energy derives from oxidation of carbohydrates and fatty acids in the mitochondria. The common metabolic product of sugars

and fats is acetyl-CoA, which enters the Krebs cycle. Oxidation of one molecule of acetyl-CoA results in the reduction of three molecules of NAD and one of FAD. These reducing equivalents flow down a chain of carriers (Fig. 43-4) through a series of oxidation–reduction events. The final hydrogen acceptor is molecular oxygen, and the product is water. The released energy "charges" the inner mitochondrial membrane, converting the mitochondrion into a veritable biological battery. This oxidation process is coupled to ATP synthesis from ADP and inorganic phosphate (Pi), catalyzed by mitochondrial ATPase (DiMauro & Bonilla, 2004; DiMauro & Schon, 2003). Considering the enormous amount of information collected since 1960 on mitochondrial structure and function, it is surprising that diseases of terminal mitochondrial metabolism, that is, the Krebs cycle and respiratory chain, have attracted the attention of clinical investigators only recently.

Initial clues that some diseases might be due to mitochondrial dysfunction came from electron-microscopic studies of muscle biopsies showing fibers with increased numbers of structurally normal or abnormal mitochondria. These fibers have a "ragged red" appearance in the modified Gomori trichrome stain. Because the diagnosis was based on mitochondrial changes in muscle biopsies, these disorders were initially labeled mitochondrial myopathies. It soon became apparent, however, that many mitochondrial diseases with ragged red fibers (RRF) were not confined to skeletal muscle but were multisystem disorders. In these patients, the clinical picture is often dominated by signs and symptoms of muscle and brain dysfunction, probably due to the great dependence of these tissues on oxidative metabolism. This group of disorders, often called mitochondrial encephalomyopathies, includes four more common syndromes (Table 43-4) (DiMauro & Bonilla, 2004; DiMauro & Schon, 2003).

The first, Kearns-Sayre syndrome (Malm et al., 1996), is characterized by childhood onset of progressive external ophthalmoplegia and pigmentary degeneration of the retina. Heart block, cerebellar syndrome or high CSF protein may also appear. Almost all cases are sporadic. The second syndrome, myoclonus epilepsy with ragged red fibers (MERRF), is characterized by myoclonus, ataxia, weakness and generalized seizures. The third syndrome, mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS), affects young children, who show stunted growth, episodic vomiting and headaches, seizures and recurrent cerebral insults resembling strokes and causing hemiparesis, hemianopsia or cortical blindness. The fourth syndrome, neuropathy, ataxia, retinitis pigmentosa/maternally inherited Leigh syndrome (NARP/MILS) comes in two flavors: NARP is usually seen in young adults and is characterized by retinitis pigmentosa, dementia, seizures, ataxia, proximal weakness, and sensory neuropathy, whereas MILS affects infants and children (often in the same family) and is a devastating encephalomyopathy with the neuroradiological and neuropathological symmetrical CNS lesions of Leigh syndrome. KSS is almost always a sporadic condition whereas MERRF, MELAS, and NARP/MILS are transmitted by non-Mendelian, maternal inheritance. All four conditions are due to mutations in mitochondrial DNA (mtDNA); KSS is associated with single, large-scale mtDNA deletions; MERRF is typically due to a point mutation (m.8334A > G) in the tRNALys; MELAS is most commonly due to the m.3243A > G mutation in the tRNALeu(UUR), whereas NARP/MILS is caused by mutations in the ATPase 6 gene. The different severity of NARP and MILS is due to different mutation loads, much higher in MILS than in NARP (DiMauro & Bonilla, 2004; DiMauro & Schon, 2003).

Mitochondrial DNA is inherited maternally

What makes mitochondrial diseases particularly interesting from a genetic point of view is that the mitochondrion has its own DNA (mtDNA) and its own transcription and translation processes. The mtDNA encodes only 13 polypeptides; nuclear DNA (nDNA) controls the synthesis of 90 to 95% of all mitochondrial proteins. All known mitochondrially encoded polypeptides are located in the inner mitochondrial membrane as subunits of the respiratory chain complexes (Fig. 43-4), including seven subunits of complex I; the apoprotein of cytochrome b; the three larger subunits of cytochrome *c* oxidase, also termed complex IV; and two subunits of ATPase, also termed complex V.

In the formation of the zygote, all mitochondria are contributed by the ovum. Therefore, mtDNA is transmitted by maternal inheritance in a vertical, non-Mendelian fashion. Strictly maternal transmission of mtDNA has been documented in humans by studies of restriction fragment length polymorphisms (RFLPs) in DNA from platelets. As exemplified by the disorders outlined above, diseases caused by mutations of mtDNA are also transmitted by maternal inheritance: an affected mother ought to pass the disease to all of her children, were it not for the "threshold effect," which is described later, but only her daughters can transmit the trait to subsequent generations (DiMauro & Bonilla, 2004; DiMauro & Schon, 2003). Characteristics that distinguish maternal from Mendelian inheritance include the following:

1. The number of affected individuals in subsequent generations should be higher than in autosomal dominant disease, again, were it not for the "threshold effect" (see below).
2. Inheritance is maternal, as in X-linked diseases, but children of both sexes are affected.
3. Because there are hundreds or thousands of copies of mtDNA in each cell, the phenotypic expression of a mitochondrially encoded gene depends on the relative proportions of mutant and wild-type mtDNAs within a cell; this is termed the "threshold effect."
4. Because mitochondria replicate more often than do nuclei, the relative proportion of mutant and wild-type mtDNAs may change within a cell cycle.
5. At the time of cell division, the proportion of mutant and wild-type mtDNAs in the two daughter cells can shift, thus giving them different genotypes and, possibly, different phenotypes, a phenomenon called mitotic segregation.

As described above, maternal inheritance has been documented in diseases due to point mutations of mtDNA, while most diseases due to mtDNA deletions or duplications are sporadic.

TABLE 43-4 Clinical Features of Mitochondrial Diseases Associated with mtDNA Mutations

Tissue	Symptom/sign	*Δ-mtDNA*		*tRNA*		*ATPase6*	
		KSS	Pearson	MERRF	MELAS	NARP	MILS
CNS	Seizures	−	−	+	+	−	+
	Ataxia	+	−	+	+	+	±
	Myoclonus	−	−	+	±	−	−
	Psychomotor retardation	−	−	−	−	−	+
	Psychomotor regression	+	−	±	+	−	−
	Hemiparesis/hemianopia	−	−	−	+	−	−
	Cortical blindness	−	−	−	+	−	−
	Migraine-like headaches	−	−	−	+	−	−
	Dystonia	−	−	−	+	−	+
PNS	Peripheral neuropathy	±	−	±	±	+	−
Muscle	Weakness	+	−	+	+	+	+
	Ophthalmoplegia	+	±	−	−	−	−
	Ptosis	+	−	−	−	−	−
Eye	Pigmentary retinopathy	+	−	−	−	+	±
	Optic atrophy	−	−	−	−	±	±
	Cataracts	−	−	−	−	−	−
Blood	Sideroblastic anemia	±	+	−	−	−	−
Endocrine	Diabetes mellitus	±	−	−	±	−	−
	Short stature	+	−	+	+	−	−
	Hypoparathyroidism	±	−	−	−	−	−
Heart	Conduction block	+	−	−	±	−	−
	Cardiomyopathy	±	−	−	±	−	±
Gastrointestinal	Exocrine pancreas dysfunction	±	+	−	−	−	−
	Intestinal pseudo-obstruction	−	−	−	−	−	−
ENT	Sensorineural hearing loss	−	−	+	+	±	−
Kidney	Fanconi's syndrome	±	±	−	±	−	−
Lab	Lactic acidosis	+	+	+	+	−	±
	Muscle bx: RRF	+	±	+	+	−	−
Inheritance	Maternal	−	+	+	±	+	+
	Sporadic	+	−	−	±	−	−

Boxes highlight typical features of different syndromes, except for maternally inherited Leigh syndrome (MILS), which is defined on the basis of neuroradiologic or neuropathologic criteria. Pearson's syndrome is a hematological disorder of infancy characterized by sideroblastic anemia and exocrine pancreas dysfunction. −, absent; +, present; Δ-mtDNA, deleted mtDNA; KSS, Kearns–Sayre syndrome; MELAS, mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes; MERRF, myoclonus epilepsy with ragged-red fibers; MILS, maternally inherited Leigh syndrome; NARP, neuropathy, ataxia and retinitis pigmentosa.

The genetic classification of mitochondrial diseases divides them into three groups

Defects of mtDNA include point mutations and deletions or duplications. From a biochemical point of view, these disorders will be associated with dysfunction of the respiratory chain because all 13 subunits encoded by mtDNA are subunits of respiratory chain complexes. Diseases due to point mutations are transmitted by maternal inheritance, and the number has rapidly increased since the first pathogenic mtDNA mutations were described 22 years ago. The main syndromes include MERRF; MELAS; NARP/MILS (Table 43-4); and Leber hereditary optic neuropathy (LHON), a disorder causing blindness in young adult men. Diseases due to deletions or duplications are usually sporadic, for reasons that are not completely clear. They include, besides KSS (Table 43-4), isolated progressive external ophthalmoplegia and Pearson's syndrome, a usually fatal infantile disorder dominated by sideroblastic anemia and exocrine pancreas dysfunction.

Defects of nuclear DNA

Defects of nuclear DNA also cause mitochondrial diseases. As mentioned above, the vast majority of mitochondrial proteins are encoded by nDNA, synthesized in the cytoplasm and "imported" into the mitochondria, through a complex series of steps. Diseases can be due to mutations in genes

encoding respiratory chain subunits ("direct hits"), ancillary proteins controlling the proper assembly of the respiratory chain complexes ("indirect hits"), proteins controlling the importation machinery, proteins controlling the lipid composition of the inner membrane, or proteins controlling mitochondrial movement, fusion and fission (mitochondrial dynamics). All these disorders will be transmitted by Mendelian inheritance. From a biochemical point of view, all areas of mitochondrial metabolism can be affected (see below).

Defects of communication between nDNA and mtDNA can also cause mitochondrial diseases

The nDNA controls many functions of the mtDNA, including its replication and translation. It is, therefore, predictable that mutations of nuclear genes controlling these functions could cause alterations in the mtDNA. Three groups of human diseases are due to faulty intergenomic communication (Spinazzola et al., 2009). The first is associated with multiple mtDNA deletions and is characterized clinically by ptosis, progressive external ophthalmoplegia (PEO), weakness of limb and respiratory muscles, peripheral neuropathy, parkinsonism, and—often—psychiatric disorders. Transmission is more commonly autosomal dominant than recessive. Mutations in four genes have been associated with autosomal dominant PEO syndromes (*ANT1*, encoding adenine nucleotide transporter 1; *PEO1*, encoding the Twinkle helicase, *POLG*, encoding the only mtDNA polymerase; and *OPA1*, encoding a guanosine triphosphatase involved in mitochondrial fusion), whereas recessive PEO syndromes have been associated with mutations in *POLG* and in *TYMP*, which encodes the matrix enzyme thymidine phosphorylase. *TYMP* mutations cause a multisystem disorder called mitochondrial neurogastrointestinal encephalomyopathy, or MNGIE (DiMauro & Bonilla, 2004; DiMauro & Schon, 2003). The second group of disorders is associated with mtDNA depletion in one or more tissues, more commonly in muscle (myopathic form) or in liver and brain (hepatocerebral form). Depending on the tissues affected and the severity of the mtDNA decrease, the clinical picture can be a rapidly fatal congenital myopathy, a slightly more benign myopathy of childhood, a fatal hepatopathy, or a multisystem disorder. Transmission is autosomal recessive, and to date nine genes have been associated with mtDNA depletion syndromes: *DGUOK*, *TK2*, *POLG*, *SUCLA2*, *SUCLG*, *PEO1*, *RRM2B*, *TYMP* and *MPV17* (Spinazzola et al., 2009). It is notable that some gene defects (*POLG*, *PEO1*, and *TYMP*) can cause both mtDNA depletion and multiple deletions. This is not surprising when one considers that all of these genes except *MPV17*, are involved in the homeostasis of the mitochondrial nucleoside/nucleotide pool, which is crucial for mtDNA maintenance.

Defects in genes controlling mtDNA translation

The last—and rapidly expanding—group of disorders is due to defects in genes controlling mtDNA translation, including the transcription of the 12S rRNA (*MRP16*), the mitochondrial translation elongation factor G1 (*GFM1*), the pseudouridylation of tRNA (*PUS1*), and the structure and function of mtDNA synthetases (*DARS2*, *RARS2*). The associated disorders are usually infantile hepatocerebral syndromes with multiple respiratory chain activity defects but no evidence of mtDNA mutations or depletion. However, defects in pseudourydilation are associated with mitochondrial myopathy and sideroblastic anemia (MLASA) (Jacobs & Turnbull, 2005).

The biochemical classification of mitochondrial DNA is based on the five major steps of mitochondrial metabolism

These steps are illustrated in Figure 43-4 and divide mitochondrial diseases into five groups: (1) defects of mitochondrial transport, (2) defects of substrate utilization, (3) defects of the Krebs cycle, (4) defects of oxidation—phosphorylation coupling and (5) defects of the respiratory chain.

All disorders except those in group 5 are due to defects of nDNA and are transmitted by Mendelian inheritance. Disorders of the respiratory chain can be due to defects of nDNA or mtDNA. Usually, mutations of nDNA cause isolated, severe defects of individual respiratory complexes, whereas mutations in mtDNA or defects of intergenomic communication cause variably severe, multiple deficiencies of respiratory chain complexes. The description that follows is based on the biochemical classification.

Defects of mitochondrial transport

Defects of mitochondrial transport interfere with the movement of molecules across the inner mitochondrial membrane, which is tightly regulated by specific translocation systems. The carnitine cycle is shown in Figure 43-3 and is responsible for the translocation of acyl-CoA thioesters from the cytosol into the mitochondrial matrix. The carnitine cycle involves four elements: (1) the plasma membrane carnitine-transporter system (OCTN2), (2) CPT I, (3) the carnitine–acyl carnitine translocase system in the inner mitochondrial membrane, and (4) CPT II. Genetic defects have been described for each of these four steps, as discussed previously (DiDonato & Taroni, 2008; Ohkuma et al., 2009).

Defects of substrate utilization

Pyruvate dehydrogenase (PDH) deficiency can cause alterations of pyruvate metabolism, as can defects of pyruvate carboxylase, as discussed earlier. Several hundred patients have been described with a disturbance of the PDH complex (PDHC) (De Meileir, 2002). The clinical picture includes several phenotypes ranging from a severe, devastating metabolic disease in the neonatal period to a benign, recurrent syndrome in older children. There is considerable overlap clinically and biochemically with other disorders (see below).

The PDHC catalyzes the irreversible conversion of pyruvate to acetyl-CoA (Fig. 43-4) and is dependent on thiamine and lipoic acid as cofactors (see Ch. 11). The complex has five enzymes: three subserving a catalytic function and two subserving a regulatory role. The catalytic components include PDH, E1; dihydrolipoyl transacetylase, E2; and dihydrolipoyl dehydrogenase, E3. The two regulatory enzymes include

PDH-specific kinase and phospho-PDH-specific phosphatase. The multienzyme complex contains nine protein subunits, including protein X. Protein X anchors the E3 component to the E2 core of the complex. The E1 α subunit is encoded by a gene on the short arm of the X chromosome and a gene on chromosome 4. The E1 β subunit is encoded by a gene on chromosome 3, the E2 component is encoded by a gene on chromosome 11, and the E3 component is encoded by a gene on chromosome 7. Biochemical defects have been documented for the E1 α subunit, E2, E3, protein X and the phospho-PDH-specific phosphatase. The great majority of cases involve a mutation defect of the E1 α subunit (De Meileir, 2002). Both genders are equally represented despite the location of the E1 α-subunit gene on the X chromosome.

The most devastating phenotype of PDH deficiency presents in the newborn period. The majority of patients are male and critically ill with a severe metabolic acidosis. There is an elevated blood and CSF lactate concentration and associated elevations of pyruvate and alanine. The CSF lactate is more consistently elevated, giving rise to the descriptive term "cerebral lactic acidosis." These patients have seizures, failure to thrive, optic atrophy, microcephaly and dysmorphic features. Multiple brain abnormalities have been described, including dysmyelination of the cortex, cystic degeneration of the basal ganglia, ectopic olivary nuclei, hydrocephalus and partial or complete agenesis of the corpus callosum. A less devastating phenotype presents in early infancy. These patients demonstrate the histopathological features of Leigh syndrome. Other patients affected in infancy survive with a chronic neurodegenerative syndrome manifested by mental retardation, microcephaly, recurrent seizures, spasticity, ataxia and dystonia.

Mutations involving the E1 α subunit behave clinically like an X-linked dominant condition. These mutations usually are lethal in boys during early infancy. The clinical spectrum in the heterozygous girl is more varied, ranging from a devastating condition in early infancy to a mild chronic encephalopathy with mental retardation. The least symptomatic woman may give birth to affected male and female progeny and pose a significant problem in clinical diagnosis and genetic counseling.

Treatment is largely symptomatic, and the prognosis ranges from dismal to guarded. Thiamine, lipoic acid, ketogenic diet and physostigmine have been tried in different concentrations and doses with equivocal results. Some patients with periodic ataxia resulting from PDHC deficiency may respond to acetazolamide.

Glutaric aciduria type II, which is a defect of β-oxidation, may affect muscle exclusively or in conjunction with other tissues. Glutaric aciduria type II, also termed multiple acyl-CoA dehydrogenase deficiency (Fig. 43-3), usually causes respiratory distress, hypoglycemia, hyperammonemia, systemic carnitine deficiency, nonketotic metabolic acidosis in the neonatal period and death within the first week. A few patients with onset in childhood or adult life showed lipid-storage myopathy, with weakness or premature fatigue (DiDonato & Taroni, 2008). Short-chain acyl-CoA deficiency (Fig. 43-3) was described in one woman with proximal limb weakness and exercise intolerance. Muscle biopsy showed marked accumulation of lipid droplets. Although no other tissues were studied, the defect appeared to be confined to skeletal muscle, suggesting the existence of tissue-specific isozymes.

Defects of the Krebs cycle

Fumarase deficiency was reported in children with mitochondrial encephalomyopathy. Usually, there is developmental delay since early infancy, microcephaly, hypotonia and cerebral atrophy, with death in infancy or early childhood. The laboratory hallmark of the disease is the excretion of large amounts of fumaric acid and, to a lesser extent, succinic acid in the urine. The enzyme defect has been found in muscle, liver and cultured skin fibroblasts (De Meileir, 2002).

Defects of oxidation—phosphorylation Coupling

The best-known example of such a defect is Luft disease, or nonthyroidal hypermetabolism. Only two patients with this condition have been reported. Family history was noncontributory in both cases. Symptoms started in childhood or early adolescence with fever, heat intolerance, profuse perspiration, resting tachypnea and dyspnea, polydipsia, polyphagia and mild weakness. The basal metabolic rate was markedly increased in both patients, but all tests of thyroid function were normal. Muscle biopsies showed ragged red fibers and proliferation of capillaries. Other tissues were morphologically normal. Studies of oxidative phosphorylation in isolated muscle mitochondria from both patients showed maximal respiratory rate even in the absence of ADP, an indication that respiratory control was lost. Respiration proceeded at a high rate independently of phosphorylation, and energy was lost as heat, causing hypermetabolism and hyperthermia (DiMauro & Bonilla, 2004; DiMauro & Schon, 2003).

Abnormalities of the respiratory chain

Abnormalities of the respiratory chain are increasingly identified as the hallmark of "mitochondrial diseases" or "mitochondrial encephalomyopathies". They can be identified on the basis of polarographic studies showing differential impairment in the ability of isolated intact mitochondria to use different substrates. For example, defective respiration with NAD-dependent substrates, such as pyruvate and malate, but normal respiration with FAD-dependent substrates, such as succinate, suggests an isolated defect of complex I (Fig. 43-4). However, defective respiration with both types of substrates in the presence of activity of normal cytochrome c oxidase, also termed complex IV, localizes the lesions to complex III (Fig. 43-4). Because frozen muscle is much more commonly available than fresh tissue, electron transport is usually measured through discrete portions of the respiratory chain. Thus, isolated defects of NADH—cytochrome c reductase, or NADH-coenzyme Q (CoQ) reductase suggest a problem within complex I, while a simultaneous defect of NADH and succinate—cytochrome c reductase activities points to a biochemical error in complex III (Fig. 43-4). Isolated defects of complex III can be confirmed by measuring reduced CoQ-cytochrome c reductase activity.

Abnormalities of the respiratory chain: defects of complex I

Known or presumed nuclear defects of complex I usually affect infants or children. Some have fatal infantile multisystem

disorders, characterized by severe congenital lactic acidosis, psychomotor delay, diffuse hypotonia and weakness, cardiopathy and cardiorespiratory failure. Some have the neuroradiological and neuropathological features of Leigh syndrome. Interestingly, a handful of mutations in nuclear genes encoding complex I subunits have been identified in these children, making prenatal diagnosis possible for some families.

Complex I deficiency due to mtDNA mutations (seven subunits of complex I are encoded by mtDNA) can be divided into encephalomyopathies and myopathies. The most important encephalomyopathy is Leber hereditary optic neuropathy (LHON), characterized by acute or subacute loss of vision due to severe bilateral optic atrophy, with onset usually between 18 and 30 years and marked predominance in men. Three mutations (in ND1, ND4 and ND6) are considered "primary," that is, pathogenic in and by themselves. Increased numbers of mutations in ND5 have been associated with clinical phenotypes resembling MELAS, Leigh syndrome, MERRF or overlapping syndromes. As expected, all these disorders are inherited maternally (DiMauro & Bonilla, 2004; DiMauro & Schon, 2003).

Myopathies have been described in several sporadic cases with lactic acidosis, COX-positive RRF in muscle and isolated complex I deficiency, and have been attributed to various pathogenic mutations in ND genes. The sporadic nature of these myopathies suggests that the ND mutations are de novo, arising spontaneously in myogenic stem cells after germ-layer differentiation (somatic mutations) (DiMauro et al., 2004; DiMauro et al., 2003).

Abnormalities of the respiratory chain: defects of complex II

These have not been fully characterized in the few reported patients, and the diagnosis has often been based solely on a decrease of succinate—cytochrome c reductase activity (Fig. 43-4). However, partial complex II deficiency was documented in muscle and cultured fibroblasts from two sisters with clinical and neuroradiological evidence of Leigh syndrome, and molecular genetic analysis showed that both patients were homozygous for a point mutation in the flavoprotein subunit of the complex (Bourgeron et al., 1995). This was the first documentation of a molecular defect in the nuclear genome associated with a respiratory chain disorder.

Abnormalities of the respiratory chain: coenzyme $Q1_0$ (CoQ_{10}) deficiency

This mitochondrial encephalomyopathy has three main clinical presentations. A predominantly myopathic form is characterized by the triad of exercise intolerance, recurrent myoglobinuria, and CNS involvement. A more frequent ataxic form is dominated by ataxia and cerebellar atrophy, variously associated with weakness, developmental delay, seizures, pyramidal signs, and peripheral neuropathy, often simulating spinocerebellar atrophy. A third variant presents with fatal infantile encephalomyopathy and renal involvement (nephrosis). The biochemical defect (or defects) involves different steps in the biosynthesis of CoQ_{10}, and molecular defects have been documented in 5 of the approximately 12 genes involved in CoQ_{10} biosynthesis. Diagnosis is important because all patients—and especially those with the myopathic and infantile forms—benefit from CoQ_{10} supplementation (Quinzii & Hirano, 2010).

Abnormalities of the respiratory chain: defects of complex III

As defects of complex I, these can be due to nDNA mutations or to mtDNA mutations. The only nuclear defect described thus far does not affect a complex III subunit, but an ancillary protein needed for proper assembly, BCS1L. Mutations in *BCS1L* gene can cause a Leigh syndrome-like disorder or a fatal infantile disease called GRACILE (growth retardation, aminoaciduria, cholestasis, iron overload, lactacidosis and early death).

Mutations in the only mtDNA-encoded subunit of complex III (cytochrome *b*) can cause multisystem disorders or—more commonly—isolated myopathies, manifested by exercise intolerance with or without exercise-related myoglobinuria. Patients with myopathy are almost invariably sporadic, suggesting that the cytochrome *b* mutations are somatic. Muscle biopsies in these patients show COX-positive RRF and isolated complex III deficiency.

Abnormalities of the respiratory chain: defects of complex IV

These disorders, also termed cytochrome *c* oxidase deficiency, have clinical phenotypes that fall into two main groups: one in which myopathy is the predominant or exclusive manifestation and another in which brain dysfunction predominates (Fig. 43-4). In the first group, the most common disorder is fatal infantile myopathy, causing generalized weakness, respiratory insufficiency and death before age 1 year. There is lactic acidosis and renal dysfunction, with glycosuria, phosphaturia and aminoaciduria, also termed DeToni-Fanconi-Debre syndrome. The association of myopathy and cardiopathy in the same patient and myopathy and liver disease in the same family has also been described.

In patients with pure myopathy, COX deficiency is confined to skeletal muscle, sparing heart, liver and brain. The amount of immunologically reactive enzyme protein is markedly decreased in muscle by enzyme-linked immunosorbent assay (ELISA) and by immunocytochemistry of frozen sections. "Benign" (better defined as "reversible") infantile mitochondrial myopathy, in contrast, has been described in children born with severe myopathy and lactic acidosis, who then improve spontaneously and are virtually normal by age 2 years. This condition is due to a reversible COX deficiency. The enzyme activity is markedly decreased, <19% of normal, in muscle biopsies taken soon after birth but returns to normal in the first year of life. Immunocytochemistry and immunotitration show normal amounts of enzyme protein in all muscle biopsies. This finding differs from the virtual lack of CRM in patients with fatal infantile myopathy and may represent a useful prognostic test. The selective involvement of one or more tissues and the reversibility of the muscle defect in the benign form suggest the existence of tissue-specific and developmentally regulated COX isoenzymes in humans. Surprisingly, however, the reversible form of COX deficiency

myopathy has been clearly attributed to a homoplasmic mutation (m.14674T > C) in the $tRNA^{Glu}$ of mtDNA (Horvath et al., 2009). While this mutation is not in doubt, the question now is what makes this homoplasmic mutation pathogenic in some but not all maternally related members of a family and what makes it tissue-specific and reversible. It is likely that nuclear modifier genes are involved but none has been identified thus far. Some patients with exercise intolerance and myoglobinuria have more conventional, heteroplasmic, somatic mutations in mtDNA-encoded genes.

Subacute necrotizing encephalomyelopathy, also termed Leigh syndrome, typifies the second group of disorders of complex IV, dominated by involvement of the CNS. Leigh syndrome usually starts in infancy or childhood and is characterized by psychomotor retardation and brainstem abnormalities (DiMauro & Bonilla, 2004; DiMauro & Schon, 2003). The pathological hallmark consists of focal, symmetrical necrotic lesions from thalamus to pons, but also involving the inferior olives and the posterior columns of the spinal cord. Microscopically, these spongy brain lesions show demyelination, vascular proliferation and astrocytosis. In these patients, COX deficiency is generalized, including cultured fibroblasts in most, but not all, cases. This may provide a useful tool for prenatal diagnosis in at least some families. Immunological studies show CRM in all tissues. Great strides have been made in our understanding of the molecular bases of COX-deficient Leigh syndrome (DiMauro & Bonilla, 2004; DiMauro & Schon, 2003). Only very recently has the first mutations been found in a gene encoding a COX subunit, but several COX-assembly genes have been involved. Most mutations causing typical Leigh syndrome are in the *SURF1* gene. Mutations in other genes often affect one other tissue besides the brain, such as the heart (*SCO2* and *COX15*), the liver (*SCO1*) or the kidney (*COX10*). Even though there is no effective therapy for these disorders, at least we can now offer prenatal diagnosis to many affected families.

Abnormalities of the respiratory chain: defects of complex V

The most important disorder is NARP/MILS, an encephalomyopathy already described above, which can present in the same family as Leigh syndrome in infants or as a milder multisystem disorder in young adults (NARP). In fact, NARP/MILS is a good example of the importance of the mutation load. When the common mutation (m.8993T > G in the ATPase 6 gene of mtDNA) is present in great abundance (>90%), the phenotype is MILS; when the same mutation is less abundant (about 70%), the phenotype is NARP. Another interesting aspect of the NARP/MILS phenotype is that it can also be caused by a distinct mutation in the same nucleotide (m.8993T > C). Both clinical and biochemical expressions are milder with the T-to-C than with the T-to-G mutation. As expected, ATP production by mitochondria isolated from cultured fibroblasts harboring almost homoplasmic levels of the m.89993T > G mutation was markedly decreased. A few other mutations in the ATPase 6 gene have been associated with Leigh syndrome or with a similar but milder condition called familial bilateral striatal necrosis (FBSN).

Mutations in one nuclear gene (ATP12), encoding an ATPase assembly protein, have been associated with complex V deficiency in an infant with congenital lactic acidosis and a rapidly fatal disorder affecting brain, liver, heart and muscle (De Meirleir et al., 2004). More common causes of complex V deficiency are mutations in the assembly gene *TMEM70*, especially in infants of Roma ethnic origin, who have multisystem symptoms, lactic acidosis and 3-methylgutaconic aciduria.

ALL ROADS LEAD TO ROME: THE RELEVANCE OF LIPID RAFTS IN THE PATHOGENESIS OF METABOLOPATHIES

Ernesto R. Bongarzone

The plasma membrane is the physical boundary of the cell. It plays fundamental roles on multiple cellular processes, including the regulation of signals in and out of the cell, the endocytosis and exocytosis of material and pathogens, the formation and stability of synapses and the myelination of axons. Many of these processes rely upon the existence of specialized plasma membrane microdomains, also known as lipid rafts. These membrane realms are highly fluid, dynamic and heterogeneous in size, composition and degree of lateral diffusion (movement) within the plasma membrane. Lipid rafts appear to form by the coalescence of molecules with specific physicochemical properties, including certain sterols (cholesterol), sphingolipids (gangliosides; sphingosines; ceramides) and a collection of associated proteins (Lingwood et al., 2010). Their planar architecture makes them "invisible" to traditional microscopic techniques, contributing to the controversy of an elusive existence.

As integral components of the membrane, lipid rafts appear to participate in multiple cellular processes that require specific architectural conformations of receptors (i.e., Patched receptor, Karpen et al. 2001), integral proteins (myelin proteolipid, Simons et al. 2000) and specific enzymatic activities (gamma secretase, Kapoor et al. 2010). Many if not all of the raft-associated functions are highly influenced by their biochemical composition. Too much or too little of any of the associated raft components will inevitably impact on the ability of these domains to convey correct signals and appropriate biophysical architecture of the membrane. It is with this idea in mind that rafts are seen as converging dynamic platforms that may be affected in multiple, unrelated metabolic diseases, triggering multiple defects.

Psychosine is a toxic sphingolipid that is thought to cause the death of oligodendrocytes in Krabbe disease (see Chapter 43). The pathogenic mechanism/s of psychosine are only

ALL ROADS LEAD TO ROME: THE RELEVANCE OF LIPID RAFTS IN THE PATHOGENESIS OF METABOLOPATHIES (*cont'd*)

partially characterized but appear to involve several pathways including phospholipases, peroxisomal function, mobilization of stored calcium, caspases, PKC and even mitochondrial function. One interpretation of the involvement of such a diverse array of pathways is that psychosine targets a "master" function and/or structure, which in turn can alter the function of multiple downstream effectors. Because of its sphingolipidic nature, psychosine was an ideal candidate molecule to study raft function in Krabbe disease. Not surprisingly, psychosine was found to accumulate and disrupt rafts in the nervous system of animal models of Krabbe disease as well as in affected patients, suggesting a consequent disruption of downstream signaling (see Ch. 43). Growing numbers of examples of raft dysfunction in unrelated inborn metabolic diseases are being reported (Walkley et al., 2000; Kosicek et al. 2010; Rakheja et al., 2004), so this may be a more common component of pathogenesis that previously suspected. Further, lipid rafts may have a role in the pathogenesis of other unrelated diseases such as viral entry infection, Parkinson disease, Alzheimer's disease and others. The growing interest in understanding the biology and behavior of lipid rafts in disease will undoubtedly reveal more relevant mechanistic functions and therapeutic targets. It is likely that further studies of these membrane conundrums will deliver radical therapies for the treatment of an array of neurological diseases.

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