CHAPTER

Lysosomal storage disorders: sphingolipidoses 10

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10.1 INTRODUCTION

Sphingolipidoses are a group of lysosomal storage diseases with defects in enzymes or activator proteins needed for the degradation of sphingolipids. Accumulation of sphingolipids in one or several organs leads to visceral, neurovisceral, or purely neurological manifestations. Niemann–Pick C disease, a primary disorder of impaired cellular lipid trafficking, is also included in this group due to secondary accumulation of sphingolipids. The defects of enzymes, accumulated substrates, and key clinical features of these diseases are summarized in Table 10.1.

10.2 OVERVIEW OF SPHINGOLIPIDS METABOLISM

Sphingolipids refer to a class of complex phospholipids with a core hydrophobic structure of ceremide, which is composed of a sphingosine head and a long-chain fatty acid chain. They are essential components of plasma membranes. De novo synthesis of sphingolipids begins in the endoplasmic reticulum (ER) and ends in the Golgi apparatus by adding carbohydrates and other modifications onto the backbone of ceremide. Sphingolipids are also constantly being recycled. Deficiencies of the lysosomal enzymes that are required for the degradation of these lipid compounds result in the sphinolipidoses. Depending on the modifying structures, sphingolipids are classified into sphingomyelins and glycosphingolipids. Sphingomyelins consist of phosphocholine or phosphoethanolmine and ceremide. Deficiency of acid sphingomyelinase results in Niemann–Pick disease type A or type B (NPD-A or NPD-B). Sphingomyelins can also secondarily accumulate in Niemann–Pick disease type C (NPD-C) due to abnormal cellular lipid trafficking. Glycosphingolipids include cerebrosides, sulfatides, globosides, and gangliosides with carbohydrate groups attached to the 1-OH position of sphingosine. Cerebrosides contain a mono sugar group (galactose or glucose) and ceremide. Galactocerebroside is primarily present in neuronal cell membranes. Galactocerebrosidase deficency, associated with Krabbe disease, is

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Table 10.1 Summary of Sphingolipidoses

Diseases	Enzyme Deficiency	Gene Symbols	Accumulating Substrates (Site of Storage)	Key Clinical Features
G_{M1} gangliosidosis	β-Galatosidase	*GLB1*	• G_{M1} gangliosides (CNS) • Oligosaccharides (CNS and visceral organs) • Keratan sulfate (Skeleton)	60% patients present during early infancy with coarse features, hepatosplenomegaly, dysostosis multiplex, macular cherry-red spots, rapid neurological deterioration, and death by 2 years. Juvenile and adult forms have slower rate of regression.
G_{M2} gangliosidoses • Tay–Sachs disease • Sandhoff disease • G_{M2} activator deficiency	• β-Hexosaminidase A • β-Hexosaminidase A&B • G_{M2} activator	• *HEXA* • *HEXB* • *GM2A*	G_{M2} gangliosides (CNS)	Infantile Tay–Sachs presents at 3–6 months with rapid and progressive cerebral and retinal degeneration, death usually by 4 years. An exaggerated startle response and macular cherry-red spots are typical signs. Juvenile and adult forms exist. Sandhoff and G_{M2} activator deficiency are clinically indistinguishable from Tay–Sachs.
Fabry disease	α-Galatosidase A	*GLA*	• Globotriaosylceramide • Galabiosylceramide • Globotriaosylsphingosine (vascular system, kidney, heart, autonomic nervous system)	Pain and acroparesthesia in the extremities, angiokeratoma and hypohidrosis in childhood. Renal failure in adulthood. Whorl-like corneal dystrophy is common in both males and females. Attenuated forms exist. Female heterozygotes may be symptomatic.
Gaucher disease	Acid β-glucosidase	*GBA*	• Glucosylceramide • Glucosylsphingosine (monocytes/macrophage system affecting liver, spleen, bone marrow, and CNS)	Type 1: Hepatosplenomegaly, hypersplenism, avascular necrosis of the hip. Childhood or adult onset. Some patients may be asymptomatic. Type 2: Hepatosplenomegaly and rapid neurodegeneration in infancy. Type 3: Intermediate between type 1 and 2.
Niemann–Pick disease type A and type B	Acid sphingomyelinase	*SMPD1*	Sphingomyelin (liver, spleen, bone marrow, lung, and CNS)	Type A: Hepatosplenomegaly and rapid neurodegeneration in infancy. Tye B: Hepatosplenomegaly in children or adults. Pulmonary involvement may occur. Hyperlipidemia is common.
Metachromatic leukodystrophy	Arylsulfatase A	*ARSA*	Sulfatides (myelins of CNS and peripheral nerves)	Progressive neurodegeneration and psychomotor regression. No organomegaly. Late-infantile, juvenile, or adult onset.
Krabbe disease	Galactosylceramidase	*GALC*	Galactosylceramide (CNS)	Extremely sensitive to sounds, light, or touch with screaming and rigidity. Progressive neurodegeneration. No organomegaly.
Farber disease	Acid ceramidase	*ASAH*	Ceramide (subtaneous tissues, joints, liver, spleen, lung, larynx, and CNS)	Painful swelling of joints, subcutaneous nodules (lipogranulomatosis) and progressive hoarseness.
Niemann–Pick disease type C	• NPC1 (95%) • NPC2 (5%)	• *NPC1* • *NPC2*	Free cholesterol (CNS and visceral organs)	Liver disease in infants. Supranuclear gaze palsy, developmental regression, ataxia, seizures in childhood. Psychotic episodes in adults.

characterized by demyelination of the central nervous system (CNS) and peripheral nervous system. Glucocerebroside or glucosylceramide is widedly distributed in different tissues and not limited to the CNS. Glucocerebrosidase or β-glucosidase deficiency is associated with Gaucher disease and has heterogenous clinical phenotypes. Sulfatides are sulfated galactosylceramides that are abundant in the myelin sheath of the central and peripheral nervous system. Excess accumulation of sulfatides is the hallmark of metachromatic leukodystrophy (MLD) due to a deficiency of arylsulfatase A or its cofactor. Globosides have more than one carbohydrate grouplinked to ceramide. Globotetraosylceramide (Gb4) is the substrate for β-hexosaminidase A and B (Sandhoff). Globotriaosylceramide (Gb3) and galabiosylceramide (Gb2) are degraded by α-galactosidase A (Fabry). Gangliosides are structurally similar to globosides with an additional sialic acid attached to a galactose residue. Defects in β-galactosidase (G_{M1} gangliosidosis) and β-hexosaminidase A and B (Tay–Sachs and Sandhoff, or G_{M2} gangliosidoses) lead to accumultion of gangliosides and neurodegeneration. As sphingolipids are mostly hydrophobic, several enzymatic reactions in the degration pathways require small nonenzymatic glycoprotein cofactors to bind to their lipid substrates to facilitate catalytic reaction. These proteins are called sphinolipid activator proteins (SAPs), including saposins and the G_{M2} activator protein. Genetic defects of these activator proteins also lead to the same clinical manifestations. The degradation pathways of sphingolipids and associated enzymes and sphingolipidoses are illustrated in Fig. 10.1.

10.3 SPHINGOLIPIDOSES

10.3.1 G_{M1} GANGLIOSIDOSIS

G_{M1} gangliosidosis (GM1) is an autosomal recessive lysosomal disorder caused by mutations in the *GLB1* gene and subsequent deficiency of β-galactosidase enzyme. This lysosomal enzyme hydrolyzes the terminal β-linked galactose residue from G_{M1} gangliosides, glycoproteins, and glycosaminoglycans. Massive storage of G_{M1} gangliosides, and less elevated asialo ganglioside derivatives are found primarily in brain in patients with GM1. Other abnormal storage materials include galactose-containing oligosaccharides derived from various sources and keratan sulfate are found in both neuronal and somatic tissues and visceral organs and excreted in the urine. Therefore, G_{M1} gangliosidosis shows combined features of a sphingolipidosis, an oligosaccharidosis, and a mucopolysaccharidosis.

More than 60% of GM1 patients present with an infantile form (type I).[1] A late infantile/juvenile form (type II) and an adult/chronic form (type III) also exist. In typical type I cases, affected infants initially present with hypotonia and then develop spasticity as the disease progresses. An exaggerated startle reflex may be present as seen in Tay–Sachs and Sandhoff diseases. Developmental delay or arrest is observed at 3–6 months of age followed by a rapid neurologic and psychomotor deterioration. A macular "cherry red" spot is found in 50% of the cases. Other symptoms include

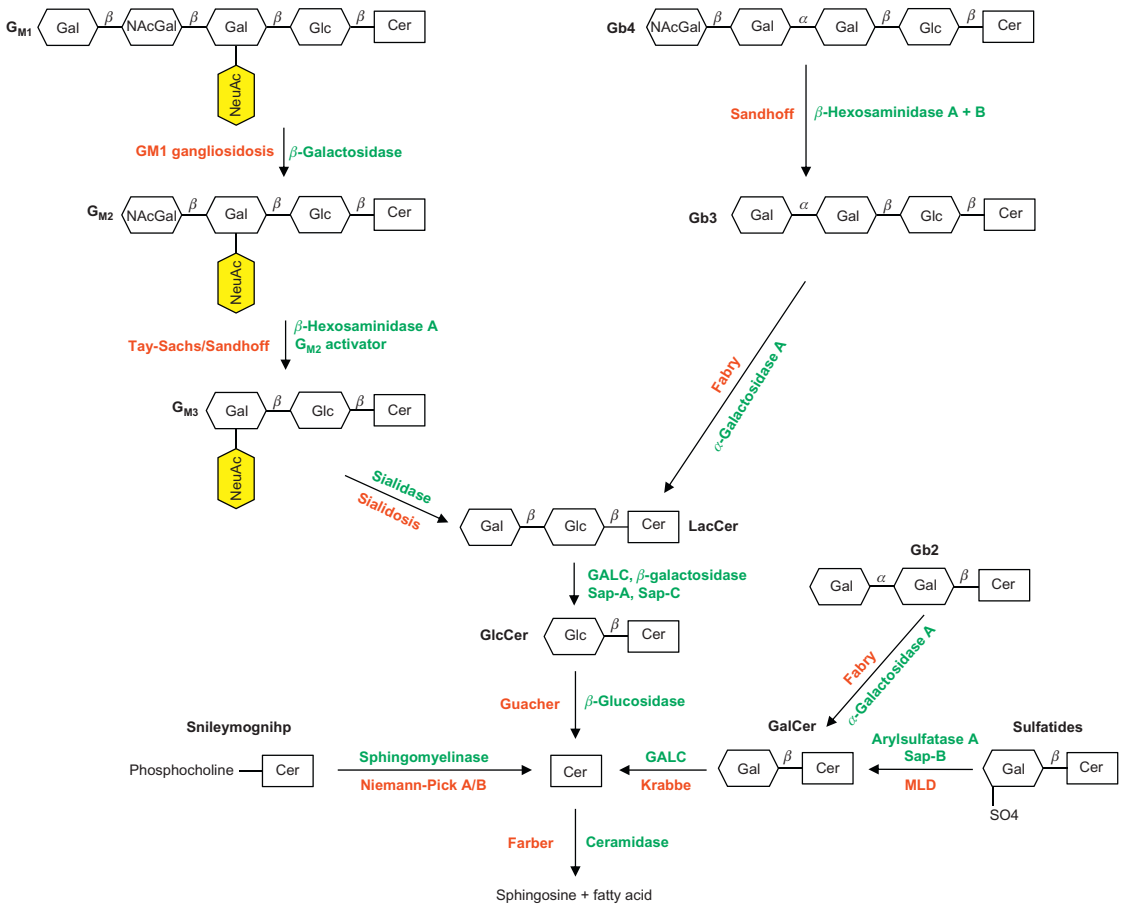

FIGURE 10.1

The degradation pathway of sphingolipids. The sphingolipids are shown in black, the enzymes are in *green*, and the diseases are in *red*. *Cer*, ceramide; *Gal*, galactose; *GALC*, galactocerebrosidase; *GalCer*, galactosylceramide; *NacGal*, N-acetylgalactosamine; *Gb2*, galabiosylceramide; *Gb3*, globotriaosylceramide; *Gb4*, globotetraosylceramide; *Glc*, glucose; *GlcCer*, glucosylceramide; G_{M1}, G_{M1} ganglioside; G_{M2}, G_{M2} ganglioside; G_{M3}, G_{M3} ganglioside; *LacCer*, lactosylceramide; *MLD*, metachromatic leukodystrophy; *NeuAc*, N-acetylneuraminic acid or sialic acid; *Sap*, saposin.

dysmorphic facial features, hepatosplenomegaly, and skeletal involvement (dysostosis multiplex). Death usually occurs by 2 years of age. Type II disease usually presents between 7 months and 3 years of life with neurocognitive and psychomotor regression. The disease progression is slower and hepatic and splenic involvement and cherry-red macular changes are less common than in type I. Type III is characterized by onset between the second and third decades of life with extrapyramidal signs (dystonia, ataxia, or speech disturbances) due to local deposition of gangliosides in the caudate nucleus. Hepatosplenomegaly and cherry-red spots are not generally present in type III.[1,2]

Urinary oligosaccharide (OS) analysis shows elevated galactose-containing OS either by qualitative visualization by thin-layer chromatography (TLC)[3] or mass profile by MALDI TOF with details of structural information.[4] Keratan sulfate excretion can also be elevated in patients with GM1; however, the amount is much lower than that seen in patients with Morquio syndrome (MPS Type IV). Demonstration of deficient β-galactosidase activity in leukocytes or cultured fibroblasts can establish a diagnosis of G_{M1} gangliosidosis. The residual enzyme activity correlates inversely with disease severity.[5] β-Galactosidase activity in leukocytes or cultured fibroblasts are severely deficient in infantile and junvenile forms (<5% of normal), and higher residual activity (5–10% of normal) is seen in patients with adult form G_{M1} gangliosidosis and Morquio B syndrome. β-Galactosidase activity is also deficient in galactosialidosis, where combined deficiencies of β-galactosidase and neuraminidase are present due to primary defects in the protective protein/cathepsin A (PPCA) that is shared by both enzymes. For this reason, it is recommended that neuraminidase activity be measured in the patients with β-galactosidase deficiency to rule out galactosialidosis.

Currently treatment is limited to supportive and symptomatic management.

10.3.2 G_{M2} GANGLIOSIDOSES (TAY–SACHS DISEASE, SANDHOFF DISEASE, AND GM2 ACTIVATOR PROTEIN DEFICIENCY)

The G_{M2} gangliosidoses are a group of lysosomal storage disorders that are characterized by deficiency of β-hexoaminidase A and accumulations of G_{M2} gangliosides and other glycolipids in the lysosomes, mainly in the neurons. Two lysosomal β-hexosaminidase isoenzymes exist, Hex A ($\alpha\beta$) and Hex B ($\beta\beta$). The α- and β-subunits of these two isozymes are encoded by two different genes, *HEXA* and *HEXB*, respectively. Both Hex A and Hex B hydrolyze the β-linked terminal nonreducing sugars *N*-acetylgalactosamine (GalNAc) and *N*-acetylglucosamine (GlcNAc) from glycolipids, glycoproteins, oligosaccharides, and glycosaminoglycans. However, the pathologic substrate G_{M2} gangliosides are almost exclusively cleaved by the Hex A enzyme in the presence of G_{M2} activator. Tay–Sachs disease is caused by a deficiency of the Hex A enzyme due to mutations in the *HEXA* gene. Sandhoff disease is caused by deficiencies of both Hex A and Hex B due to mutations in the *HEXB* gene. G_{M2} activator deficiency is caused by mutations in the *GM2A* gene and functional Hex A deficiency with similar clinical features to Tay–Sachs disease but normal Hex A activity in blood.

Infantile Tay–Sachs disease is the prototype of GM2 gangliosidosis. Patients with infantile Tay–Sachs disease present with progressive cerebral and retinal degeneration that initially becomes clinically apparent around 3–6 months of age. The symptoms include weakness, loss of motor skills, decreased attentiveness and increased startle response, and cherry-red spots on the macula. The disease is rapidly progressing with blindness, rigidity, and decerebrate posturing by 12–18 months followed by death, usually from aspiration and pneumonia, by the age of 4 years. Infantile Sandhoff disease is clinically indistinguishable from Tay–Sachs disease, although the deficiency of Hex B results in additional substrate storage of globosides and oligosaccharides. Hepatosplenomegaly, skeletal abnormalities, and other somatic changes may occur in Sandhoff disease, which are not commonly seen in Tay–Sachs disease. G_{M2} activator deficiency is also almost clinically identical to Tay-Sachs disease in its presentation and course.

The subacute or juvenile form of G_{M2} gangliosidosis presents between 2 and 10 years of age with development of ataxia, lack of coordination, dystonia, dementia, progressive seizures, and spasticity. Death usually occurs in the second decade of life. The chronic or adult form of G_{M2} gangliosidosis can have its onset from childhood to adulthood with slower rate of disease progression. Intelligence is normally intact. Neurological symptoms include dystonia, ataxia, and psychosis.

Enzyme analyses are crucial for the diagnosis of Tay–Sachs and Sandhoff diseases. The enzyme assay can be performed using serum (or plasma), white blood cells, chorionic villi, amniotic fluid, amniocytes, and other tissues. 4-Methylumbelliferyl-2-acetamido-2-deoxy-β-D-glucopyranoside (4-MUG) is the most commonly used artificial substrate that measures total β-hexosaminidase activity (Hex A and Hex B) and percentage of Hex A (Hex A%) after a two- to three-hour heat inactivation of the thermolabile Hex A. Synthetic sulfated substrate 4-methylumbelliferyl-6-sulfo-2-acetamido-2-deoxy-β-D-glucopyranoside (4-MUGS) is used to measure the specific Hex A activity. Tay–Sachs disease is characterized by absent or extremely low specific Hex A activity and a very low Hex A% (<10%) with normal or even elevated Hex B activity. Sandhoff disease has absent or extremely low total β-hexosaminidase activity with a very high Hex A% (90–100%). The heat inactivation enzyme analysis with 4MUG substrate has been the primary method for carrier testing of Tay–Sachs disease in non-Jewish populations with a detection rate of >98%. Carriers of Tay–Sachs disease have an intermediate Hex A% (20–50%) that can be discriminated from non-carriers (60–65% ± 5%). When screening for Tay–Sachs carriers, Sandhoff carriers can also be identified by a characteristic high Hex A% activity (≥75–80%) and relatively low total activity.[6]

Molecular analysis of the *HEXA* gene also plays important role in the diagnosis of Tay–Sachs disease. Three mutations c.1274_1277dupTATC (1278+ TATC), c.1421+ 1G> C (IVS12+ 1G> C), and c.805G> A (p.G269S) account for more than 98% disease causing alleles in Ashkenizi Jewish individuals.[7,8] Other founder mutations include c.1073+ 1G> A (IVS9+ 1G> A) in the Irish[9] and g.2644_10588del (del7.6kb) in French Canadians.[10] More than 180 mutations in the *HEXA* gene have been reported to date in Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/).

Most of the mutations are associated with infantile Tay–Sachs disease. The residual Hex A activity correlates inversely with the severity of the disease. Individuals with acute infantile Tay–Sachs usually have two null alleles. Individuals with juvenile or chronic and adult-onset Tay–Sachs are usually compound heterozygotes for a null allele and an allele that results in low residual Hex A activity, or compound heterozygotes of two alleles that result in low residual Hex A activity.[6,11,12] Missense mutations at two codons (178 and 258), c.532C> T (p.R178C), c.533G> A (p.R178H), c.533G> T (p.R178L), and c.772G> C (p.D258H), are associated with B1 variant of Tay–Sachs disease, presenting with juvenile or adult disease.[13] The B1 variants could have normal enzyme activities toward the 4-MUG substrate, however, are inactive toward natural substrates or the 4-MUGS substrate.[14] Carriers of the B1 variants may be falsely negative by the routine heat inactivation assay with the 4-MUG substrate. Two pseudo-deficiency variants c.739C> T (p.R247W) and c.745C> T (p.R249W) cause false-positive enzyme results resembling a Tay–Sachs carrier but are not associated with Tay–Sachs disease.[15] It has been reported about 35% of non-Jewish and 2% of Jewish individuals who are carriers by enzyme analysis are carriers for one of these two pseudo-deficiency alleles.[15] Therefore, combined enzyme and molecular testing are recommended by American College of Obstetrics and Gynecology (ACOG) for the accurate detection of Tay–Sachs carriers.

Sandhoff disease is a panethnic disorder, with a population carrier frequency of 1:300. The majority of the *HEXB* mutations are private mutations. A 16 kb deletion spanning the promoter region and exons 1–5 is the only common mutation and accounts for 27% of alleles in Sandhoff patients. Therefore, sequencing analysis is the best option for molecular diagnosis.

G_{M2} activator deficiency is suspected in patients who clinically appear to have Tay–Sachs but have normal Hex A and Hex B activities. G_{M2} activator deficiency is often diagnosed by demonstration of disease causing mutations in the *GM2A* gene.

Treatment of the G_{M2} gangliosidoses is limited to supportive care. Recently, pyrimethamine (PMT) has been tested as a potential pharmacological chaperone (PC) to treat late-onset G_{M2} gangliosidosis.[16] The enhancement of β-hexosaminidase activity has been demonstrated; however, the clinical safety and efficacy are still under investigation.[17,18]

10.3.3 FABRY DISEASE

Fabry disease is an X-linked recessive disorder caused by a deficiency of α-galactosidase A due to mutations in the *GLA* gene. This enzyme removes the α-galactose residue from globotriaosylceramide (Gb3) and to a lesser degree, from galabiosylceramide (Gb2). The isoenzyme α-galactosidase B or α-N-acetylgalactosaminidase also hydrolyzes the terminal galactose moiety toward artificial substrate; however, it is encoded by a separate but evolutionarily related gene (*NAGA*), the mutation of which causes a neuroaxonal dystrophy known as Schindler disease.[19] Deficiency of α-galactosidase A results in the accumulation of Gb3 in the lysosomes of endothelial, perithelial, and smooth muscle cells of the vascular system, as well as cells in the kidney, heart, eyes,

and ganglion cells of the autonomic nervous system. However, there is a missing link between the Gb3 storage and disease pathogenesis and disease progression. The deacylated substrate globotriaosylsphingosine or lyso-Gb3 has recently been recognized as a better biochemical marker of severity and progression for Fabry disease.[20]

The prevalence of Fabry disease is about 1 in 40,000–60,000 males. Males with classic Fabry disease have almost no detectable α-galactosidase A activity with disease onset in childhood or adolescence. The typical symptoms include pain and acroparesthesia in the extremities, angiokeratomas in the skin and mucous membranes, and hypohidrosis. The whorl-like corneal dystrophy (corneal verticillata) is also characteristic for Fabry disease (Fig. 10.2). Progressive deposition of glycosphingolipids in kidney results in proteinuria initially and progresses to end-stage renal disease. Pulmonary and cardiac involvements, along with cerebral vascular disease, are also known complications that often lead to premature death. Psychiatric illness (depression, bipolar disorder) and GI symptoms can also occur. Without ERT, death in males usually occurs in the third or fourth decade from renal or heart disease. Males with attenuated forms of Fabry disease have partial residual α-galactosidase A activity and later onset. These patients lack many classic features of the disease. The clinical findings are often limited to mild proteinuria and cardiac abnormalities. The c.936+ 919G> A (IVS4+ 919G> A) variant associated with late onset cardiac variant form is highly prevalent in the Taiwan Chinese population as identified through a pilot newborn screening study.[21]

Female heterozygotes have intermediate enzyme activity levels and variable presentations ranging from asymptomatic to classic Fabry disease due to random X-inactivation. The whorl-like corneal opacity is the most common feature which can be observed in approximately 70% of female carriers.[22]

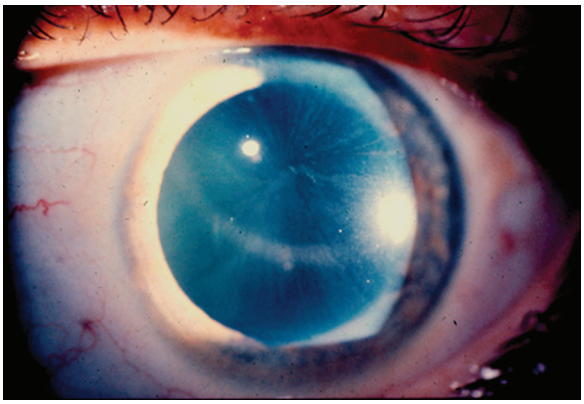

FIGURE 10.2

Corneal verticillata of Fabry disease.

Courtesy of Dr. Robert J. Desnick.

Diagnosis of classic Fabry disease is straight forward by demonstration of near absent α-galactosidase Activity in plasma, leukocytes or cultured cells and a mutation in the *GLA* gene. However, diagnosis of the attenuated form of disease and female Fabry patients can be challenging. Patients with an attenuated form of disease may have substantial residual enzyme activity and approximately 30% female carriers may have normal enzyme activity. Since these patients can potentially be missed by enzyme analysis, both enzymatic and molecular testing should be performed for diagnostic accuracy when the diagnosis is suspected. Measurement of plasma lyso-Gb3 is now considered a diagnostic test for Fabry disease, particularly for the interpretation of variants of unknown significance (VOUS) and unequivocal enzyme testing results. The α-galactosidase A activity can be measured using a flurogenic 4-methylumbelliferyl-α-D-galactopyranoside substrate or synthetic lipid substrate using tandem mass spectrometry.[23] Inhibitor of isoenzyme α-galactosidase B or α-N-acetylgalactosaminidase is usually needed for the accurate measurement of α-galactosidase A activity.

Enzyme replacement therapy (ERT) with agalsidase beta (Fabrazyme) is available for the treatment of Fabry disease. The agalsidase alfa (Replagal) is available in Europe for treating Fabry disease. ERT is of proven benefit in slowing the progression of the renal and cardiac disease associated with Fabry disease.[24] Other therapeutic options include pharmacological chaperones and substrate reduction treatment. Pain management and supportive treatment for cardiac and renal disease are also needed.

10.3.4 GAUCHER DISEASE

Gaucher disease is one of the most common lysosomal storage diseases with a prevalence of 1:40,000 to 1:50,000 in the general population and much more frequent (as high as 1:850) in the Ashkenazi Jewish individuals due to the founder mutations. This autosomal recessive disorder is caused by mutations in the *GBA* gene and subsequent deficient activity of glucocerebrosidase (acid β-glucosidase). As a result, glycosylceramide (GL1) and deacylated substrate glycosylsphingosine (Lyso-GL1) accumulate in cells of the monocytes/macrophage system. The lipid-laden "Gaucher cells" have unique wrinkled tissue paper or crumpled silk appearance in the cytoplasm and are characteristic for Gaucher disease. Gaucher cells are especially prominent in the liver and spleen, leading to hepatosplenomegaly. Progressive accumulation of Gaucher cells in the bone marrow results in bone disease including osteopenia, osteosclerosis, and osteonecrosis. In neuropathic forms of Gaucher disease, significant amounts of GL1 and Lyso-GL1 are present in the CNS.[25,26] Similar to Lyso-GB3 in Fabry disease, Lyso-GL1 appears to be correlated with disease progression and seems to be a better biomarker for the diagnosis and monitoring of Gaucher disease.[27] In addition, macrophage activation also plays a role in the pathophysiology of Gaucher disease. Several protein markers including angiotensin converting enzyme (ACE), chitotriosidase, tartrate-resistant acid phosphatase (TRAP), and the chemokine PARC/CCL18 are considered surrogate markers for lipid burden and macrophage activation and are used in monitoring progression of the disease and treatment efficacy in Gaucher disease.[25]

There are three clinical subtypes of Gaucher disease based on the involvement of CNS and rate of disease progression. Type 1 is nonneuronopathic and the most common form. Type 2 is a fatal neurodegenerative disorder of infancy in addition to the visceral involvements. Type 3 has a severity intermediate between type 1 and type 2.

Type 1 has a variable clinical phenotype, ranging from asymptomatic to severe. The classic presentations in childhood include massive hepatosplenomegaly, pancytopenia, and severe skeletal abnormalities. Some patients were initially misdiagnosed as hematologic malignancies because of pancytopenia. Despite massive hepatomegaly, liver failure is rare. Patients may also present with chronic fatigue, epistaxis, and easy bruising. The bony problems and episodic painful bone crises usually occur later than the development of visceral enlargements. Erlenmeyer flask deformity of the distal femur is a characteristic radiological finding of Gaucher bone disease. Type 2 is a more severe and fast progressing disorder with infantile onset. In addition to massive hepatosplenomegaly, neurologic abnormalities include oculomotor apraxia, cognitive impairment, psychomotor impairment, and seizures. Death usually occurs between 2 and 4 years of age. Type 3 is a more slowly progressive neuropathic form of Gaucher disease with onset in childhood or adolescence.

Recently, the association of mutations in the *GBA* gene and Parkinson disease has been studied extensively. Patients with Gaucher disease, asymptomatic patients and Gaucher carriers have a significantly increased risk for developing Parkinson disease compared to the general population.[28]

Diagnosis of Gaucher disease can be established by demonstration of reduced acid β-glucosidase activity in leukocytes or cultured fibroblasts (<15% of normal). The β-glucosidase activity can also be readily measured in dried blood spot sample for newborn screening of Gaucher disease. Molecular testing involving targeted mutational or sequence analysis of the *GBA* gene is available. Although more than 400 *GBA* mutations have been identified, four mutations, p.N409S (N370S), p.L483P (L444P), c.84dupG (84GG), and c.115+ 1G> A (IVS2+ 1), account for approximately 95% of the disease-causing alleles in the Ashkenazi Jewish population and approximately 50–60% in other populations.[26] Molecular testing of the *GBA* gene can not only confirm and diagnose Gaucher disease but can also detect Gaucher carriers for reproductive counseling. There is some genotype–phenotype correlation toward prediction of a phenotype. The p.N409S (N370S) mutation is predicative of non-neuronopathic (type 1) Gaucher disease. The p.L483P (L444P) mutation is strongly associated with the development of neuronopathic Gaucher disease: homozygosity for this mutation is associated with type 3 Gaucher disease. The p.L483P (L444P) in combination with a more severe mutation results in type 2 Gaucher disease. Measurements of biomarkers including lyso-GL1, chitotriosidase, ACE, TRAP, and CCL18 can also facilitate diagnosis.

ERT has been the standard of care for the treatment of Gaucher disease since 1992. Three enzyme preparations are available worldwide: imiglucerase (Cerezyme), velalglucerasealfa (VPRIV), and taligucerase (Elelyso). Regular ERT infusions leads to decreased plasma levels of the biochemical markers and improvement and stabilization of hematologic and visceral (liver/spleen) involvement. There has been also

improvement in frequency of bone crises and bone disease with prolonged treatment with ERT. Miglustat (Zavesca) and eliglustat are inhibitors of glucosylceramide synthase and have been approved by the US Food and Drug Administration (FDA) to treat type 1 Gaucher disease. Substrate reduction therapy (SRT) can be used alone or in combination with ERT. Pharmacological chaperones are under investigation and clinical trials for the treatment of Gaucher disease. Both SRT and pharmacological chaperones can be taken orally and may have potential to cross the blood–brain barrier for possible treatment of neuronopathic Gaucher disease. Bisphosphonates can be used as a supportive treatment for osteopenia.

10.3.5 NIEMANN–PICK A/B (NPD-A AND NPD-B)

NPD-A and NPD-B are autosomal recessive conditions caused by deficient activity of lysosomal acid sphingomyelinase (ASM), which breakdowns sphingomyelin to ceramide. As a result, sphingomyelin accumulates in the lysosomes. In addition, cholesterol and bis (monoacylglycero) phosphate and some other sphingolipids are also highly elevated. These sphingolipids play important roles in cellular signal transduction and may contribute to the disease pathophysiology in NPD-A&B. For example, sphingosine is a potent inhibitor of protein kinase C, which mediates multiple cell-signaling pathways.[29] Ceramide is a second messenger in diverse pathways involving apoptosis.[30] The deacylated sphingomyelin or sphingosylphosphorylcholine is elevated in NPD-A&B and may contribute to cell dysfunctions and apoptosis. The foam cells or Niemann–Pick cells are lipid laden macrophages found primarily in the bone marrow, liver, spleen, and lungs of all NPD-A&B patients, as well as the brains of NPD-A patients. Although Niemann–Pick cells are the histologic hallmark of NPD-A&B, histologically similarly appearing cells may be also found in GM1 gangliosidosis, Wolman disease, and cholesterol ester storage disease, and lipoprotein lipase deficiency.[31]

NPD-A and NPD-B are examples of allelic heterogeneity where different mutations in the same gene locus, *SMPD1* gene on chromosome 11p15, result in different clinical phenotypes. NPD-A is a progressive neurodegenerative disorder of infancy with less than 5% ASM activity, whereas NPD-B is a nonneuronopathic disorder with a broad range of phenotypic severity and higher residual ASM activity. Both types of NPD are panethnic; however, NPD-A is more common in the Ashkenazi Jewish population. Three founder mutations p.L304P (L302P), p.R498L (R496L), and c996delC (fsp330) account for approximately 90% of pathogenic alleles in NPD-A in this population. The other mutation, p.R610del (delR608), is found in 15–20% NPD-B patients in Western Europe and North America and is predicted to have a neuroprotective effect.

NPD-A presents within the first few months of life with massive hepatosplenomegaly, feeding difficulties, recurrent vomiting, constipation and failure to thrive. Psychomotor retardation becomes apparent after 6 months of age with hypotonia, weakness and regression in developmental milestones and at later stages, is superseded with spasticity and rigidity. Cherry-red maculae are observed in ~50% of

patients. Interstitial lung disease can result in recurrent infection and respiratory failure and death. The neurological deterioration is debilitating and most NPD-A patients die before 3 years of age.

NPD-B has more variable presentation with childhood, adolescent, or adult onset of symptoms. The clinical presentations and course are more variable. Most patients develop liver and spleen enlargement in childhood with progressive hypersplenism. Liver dysfunction is stable but, in some cases, liver disease can be severe with progression to cirrhosis. Hyperlipidemia is common with elevations of total cholesterol (elevated LDL), triglycerides, and low HDL levels. By the time of diagnosis, pulmonary infiltrations are usually present with progression to restrictive lung disease. Despite absence of neurological symptoms, cherry-red maculae are detected in 20% of NPD-B patients.[32,33]

Diagnosis of NPD-A and NPD-B is made by demonstrating deficient ASM activity (<10% of normal) in leukocytes or cultured fibroblasts. Although ASM activity is relatively higher in NPD-B, the residual ASM activity is not accurate in predicting the disease phenotype. The ASM activity can be measured in DBS specimens and there have been pilot newborn screening studies for this condition. It is important to note that individuals with the *SMPD1* mutation p.Gln294Lys may have apparently normal enzymatic activity when using artificial fluorogenic substrates.

Molecular testing involving targeted mutational or sequence analysis of the *SMPD1* gene is available. Targeted mutational analysis of common NPD-A mutations are useful in testing Ashkenazi Jewish individuals for NPD-A or their carrier status for NPD-A. Targeted mutation analysis is also effective for testing family members and for prenatal diagnosis. Analysis of other disease causing mutations can be achieved by sequence analysis of the *SMPD1* gene.

Treatment for both NPD-A and NPD-B is generally supportive. ERT with human recombinant acid sphingomyelinase (olipudase alfa) has been developed and is in clinical trial for NPD-B.[34,35]

10.3.6 METACHROMATIC LEUKODYSTROPHY

MLD is an autosomal recessive genetic condition with defective desulfation of 3-O-sulfogalactosyl containing glycolipids, particularly sulfatides. Sulfatides are mainly present in myelin sheaths of the central and peripheral nervous tissues. Degradation requires both arylsulfatase A enzyme and its activator protein saposin B. MLD is caused by the deficient activity of arylsulfatase A, which is encoded by the *ARSA* gene located on chromosome 22q13.31-qter. Sulfatides accumulate in the lysosomes and plasma membrane of myelin resulting in demyelination and characteristic deposition of metachromatic staining granules. A characteristic leukodystrophy is seen on magnetic resonance imaging (MRI) with bilateral symmetrical confluent areas of periventricular deep white matter signal change in the region of the atria and frontal horns with sparing of the subcortical U fibers (Fig. 10.3). This is described as a "butterfly" pattern. With progression, cortical and subcortical atrophy develop.[36] Excess sulfatides in non-neuronal tissues are excreted in the urine.

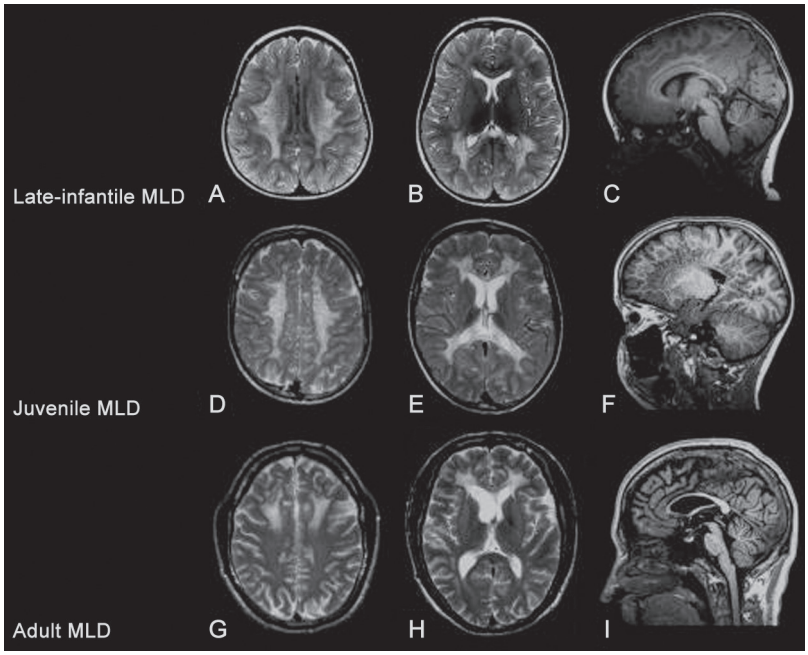

FIGURE 10.3

Axial T2-weighted (A, B, D, E, G, H) and sagittal T1-weighted (C, F, I) MR images of three patients with MLD. (A–C): 2-year-old patient with late-infantile MLD. Involvement of the periventricular white matter and centrum semiovale with parietooccipital predominance and involvement of the splenium. U fibers are spared. (D–F): 7-year-old patient with juvenile MLD. (F) Shows the typical pattern of radiating stripes with bands of normal signal intensity in between. U fibers are spared. (G–I): 28-year-old patient with adult MLD. In addition to the white matter signal abnormalities with frontal predominance, there is mild supratentorial atrophy (G, H).[36]

Saposin B deficiency and multiple sulfatase deficiency, which both have deficient function of arylsulfatase A, are different diseases with different enzymatic defects.

Late-infantile, juvenile, and adult forms have been described based on the age at disease onset. The late-infantile form accounts for 50–60% of MLD with onset between the ages of 1 to 2 years. Presenting symptoms include hypotonia, weakness, clumsiness, toe walking, and slurred speech. Regression of neurocognitive and psychomotor skills ensues (rigidity, hypertonia, hearing and vision loss, pain, peripheral neuropathy, seizures). Affected individuals eventually progress to posturing, tonic spasms, and loss of awareness with death by the age of 5 years. The juvenile form accounts for 20–30% of MLD and presents with behavioral problems, decline in school performance, gait disturbances, clumsiness, slurred speech, and incontinence before 6 years of age. Onset in adolescence may occur as well. Progression and

features of juvenile MLD are similar to but slower than late-infantile with death usually occurring before the age of 20 years. Adult-onset MLD is the least common form of MLD presenting much later in life with personality changes, decline in the ability to function in a professional setting, emotional issues, progressing to loss of coordination, peripheral neuropathy, and seizures. Disease progression is highly variable. Death, resulting from pneumonia or another related illness, usually occurs about 20 years after the onset of symptoms.

Reduced nerve conduction velocity and white matter demyelination by MRI are the major clinical findings pointed to the diagnosis of MLD. Biochemical diagnosis of MLD is based on the demonstration of deficiency of the enzyme arylsulfatase A in leukocytes or cultured fibroblasts. However, interpretation of enzyme findings could be complicated by the presence of pseudodeficiency alleles. Between 0.2% and 0.5% of normal individuals can have a considerable degree of deficiency of arylsulfatase A activity (5–15%) due to the high prevalence of the pseudodeficiency allele.[37] Therefore sequence analysis of the *ARSA* gene and urinary sulfatides analysis are needed for accurate diagnosis. In patients where there is a high clinical suspicion of disease but normal arylsulfatase A activity, saposin B deficiency should be ruled out. Diagnosis of multiple sulfatase deficiency requires testing additional sulfatases.

Treatment for MLD is palliative. Bone marrow transplantation (BMT) is used to primarily treat the CNS manifestations of the disease and may slow disease progression. BMT option remains controversial because of the high morbidity and mortality rate associated with the procedure. Long-term outcome study indicates that BMT benefits presymptomatic children with late-infantile MLD or minimally symptomatic juvenile MLD.[38] ERT has been developed and is undergoing clinical trials (rhARSA; metazyme). Hematopoietic stem cell gene therapy is another treatment modality for this devastating demyelination disorder. Preliminary outcome studies show evidence of safety and efficacy in early-onset MLD patients who received gene therapy in the presymptomatic or very early symptomatic stage.[39]

10.3.7 KRABBE DISEASE (GLOBOID CELL LEUKODYSTROPHY)

Krabbe disease is an autosomal recessive condition caused by a deficiency of lysosomal galactosylceramidase (GALC), which is encoded by the *GALC* gene on chromosome 14q31. The deficiency of GALC enzyme results in galactosylceramide storage and formation of multinucleated macrophages (globoid cells) in the CNS and almost total loss of myelin and oligodendroglia and astrocytic gliosis in the white matter. White matter disease with progressive cerebellar atrophy is identified on MRI. The peripheral nervous system is also affected in Krabbe disease. Another compound, galactsylsphingosine (psychosine), is also a substrate for the GALC enzyme and is essentially absent in normal brains but is increased in the CNS of Krabbe patients. It is postulated that psychosine plays important roles in the pathogenesis of Krabbe disease and brain psychosine levels appear to be correlated with disease severity.[40]

The incidence of Krabbe disease was estimated at 1:100,000 live births, with 90% being infantile form and 10% being late-onset form. Infantile Krabbe disease

presents with irritability, feeding difficulties, reflux, spasticity, seizures, and hypertonia at 3–6 months of age followed by rapid neurologic deterioration. Infants with Krabbe disease are extremely sensitive to sounds, light, or touch, resulting in screaming and rigidity. Blindness, deafness, and peripheral neuropathy are also common features of Krabbe disease. Organomegaly is not present. Seizures become frequent and death usually occurs before the age of two. Late-onset Krabbe disease presents between the first year of life and the fifth decade of life with a variable onset of vision disturbance/loss, weakness, and cognitive decline.[40]

Diagnosis of Krabbe disease is based on the demonstration of deficiency of the GALC enzyme and identification of the *GALC* mutations. The GALC activity can be measured in leukocytes or cultured fibroblasts by the use of radiolabeled natural substrate galactosylceramide or synthetic florescent substrate 6-hexadecanoylamino-4-methylumbelliferyl-β-D-galactopyranoside. Methods using synthetic lipid substrates and tandem mass spectrometry have recently become available. Several common disease-causing mutations are known. A 30-kb deletion (or 502T/del) is the most common mutation in populations of European ancestry accounting for approximately 40% of infantile Krabbe alleles. Two missense mutations p.T513M and p.Y551S make up for additional 0–15% of the infantile Krabbe alleles.[40,41] Another missense mutation p.G270D is common in patients with late-onset Krabbe disease.[40] Three pseudodeficiency alleles (p.R168C, p.D232N, and p.I546T) are known to cause reduced GALC activity but do not cause Krabbe disease.[40]

New York State has screened almost 2 million infants for Krabbe disease since 2006. Only 5 infants were diagnosed with infantile Krabbe disease and 46 children were identified as having moderate to high risk for later-onset disease. The incidence of infantile Krabbe disease is 1:394,000 in New York population, which is lower than the previous estimate; however, the incidence of later-onset Krabbe disease seems more prevalent than previously thought.[42,43]

At present, the treatment option for Krabbe disease is limited to hematopoietic stem cell transplantation (HSCT). This treatment remains controversial. Earlier literature reported evidence for stabilization and possible improvements in 4 patients with late-onset disease[44] and favorable outcomes in 11 presymptomatic newborns with infantile Krabbe disease[45] indicating the benefits of HSCT. However, high mortality and morbidity were reported in the patients identified by New York State newborn screening. Out of the four infantile Krabbe patients who received HSCT, two patients died from HSCT complications and two patients who survived transplantation had moderate-to-severe disability.[43]

10.3.8 FARBER DISEASE

Farber disease is a rare recessive condition of lipid metabolism associated with a deficiency of lysosomal acid ceramidase and accumulation of ceramide. Abnormal storage of ceramide is confined to the lysosomes of multiple organs and tissues leading to progressive formation of subcutaneous nodules (lipogranulomata) and granulomatous infiltrations in subcutaneous tissues and joints as well as larynx, liver, spleen, lung, heart, and CNS system.

Approximately 50% of patients described to date have classic Farber disease presenting in early infancy with the characteristic triad of painful swelling of joints, subcutaneous nodules (lipogranulomata), and progressive hoarseness. Other symptoms include feeding and breathing difficulties, poor weight gain, and intermittent fever. Neurological symptoms occur in most patients. However, the evaluation of neurological function can be difficult in Farber patients because of the joint pain. Diagnosis can almost be made from the striking appearance with cachexia, flexion contractures along with periarticular swelling and subcutaneous nodules. Demonstration of reduced or absent acid ceramidase in leukocytes or cultured fibroblast samples and two mutations in the *ASAH* gene confirm the diagnosis. Other confirmatory evidence includes the presence of perivascular aggregates of foamy histiocytes and the identification of "Farber bodies" (crescentic-shaped bodies within Schwann cells) by electron microscopy in the biopsy sample and elevated ceramide levels in cultured cells and urine.

The therapy for Farber disease is mainly palliative. BMT in two classic Farber patients resulted in improvements of somatic symptoms but was not helpful for CNS involvement.[46] BMT might be appropriate for patients with mild or no neurological symptoms.

10.3.9 NIEMANN–PICK DISEASE TYPE C

Niemann–Pick disease type C (NPD-C) is an autosomal recessive disorder with dysregulated intracellular lipid trafficking. NPD-C is panethnic with an estimated incidence of 1:100,000 to 1:120,000 live birth. NPD-C is caused by mutations in one of two genes, *NPC1* (in 95% cases) or *NPC2* (in 5% cases). *NPC1* encodes a large protein that resides in the membrane of endosomes and lysosomes and functions to allow appropriate transport of cholesterol and lipids across cell membranes. *NPC2* encodes a protein that binds to cholesterol and transports it out of the luminal space of the late endosome/lysosome into the delimiting membrane. Defects of either protein essentially trap free cholesterol and other lipids, including glycosphingolipids, in the lysosomes and result in cellular dysfunction and apoptosis.[47]

NPD-C is a highly variable neurovisceral condition that can present in infancy, childhood, or adulthood. Classically, NPD-C presents in mid-to-late childhood with vertical supranuclear gaze palsy, ataxia, dementia, and psychiatric disturbances. Seizures, dystonia, dysarthria, loss of learned speech, and dysphagia may also be present. Death occurs in the second or third decade of life, usually from aspiration pneumonia. Infants can present with liver disease, ascites, or lung disease, or may simply present with hypotonia and developmental delay. About half of NPD-C patients have neonatal cholestasis with hepatosplenomegaly and are initially suspected of having a cytomegalovirus (CMV) infection, a peroxisomal disorder or a mitochondrial disorder. Adults typically present with the classical features later in life and are more likely to present with a psychotic episode or dementia.[47,48]

The diagnosis can be suspected with the identification of foamy macrophages in blood smears or other tissue samples (liver, tonsil). Demonstration of impaired cholesterol esterification followed by filipin staining in cultured fibroblasts used to be the

key diagnostic biochemical test for NPD-C. However, the sensitivity and specificity of filipin staining test is less ideal and requires an invasive skin biopsy procedure and cell culture. It is particularly insensitive in non-classic NPD-C. The plasma biomarkers chitotriosidase and oxycholesterol can be effective in screening for NPD-C and, if abnormal, molecular genetic testing of the *NPC1* or *NPC2* gene can confirm the diagnosis.

Treatment is generally symptomatic and supportive. Miglustat has been approved in Europe, Japan, and Canada, as a therapy for treatment of progressive neurological manifestations in pediatric and adult patients with NPD-C because of the demonstration of treatment-related stabilization of key neurological manifestations.[49] Cyclodextrin is a small molecule that is designed to bind cholesterol bypassing NPC1 and NPC2 protein. It has been shown effective in treating NPD-C in animal models. Clinical trials of various forms of cyclodextrin are under way.

10.4 BIOMARKERS IN CURRENT USE

10.4.1 PLASMA CHITOTRIOSIDASE

Chitotriosidase is a biomarker of macrophage activation that can be elevated in various lipid storage lysosomal diseases including Gaucher disease, Niemann–Pick disease, galactosialidosis, and cholesteryl ester storage disease. Plasma chitotriosidase is elevated several hundred-fold in the plasma of symptomatic non-neuronopathic Gaucher patients and decreases and stabilizes with adequate ERT or SRT. It is commonly used as a surrogate marker for the assessment of disease severity and monitoring efficacy of treatment.[50,51] Chitotriosidase activity could be moderately increased in Niemann–Pick and other lipid storage diseases. However, a null *CHIT1* allele, 24 bp duplication in exon 10, is highly prevalent. About 5% of individuals are homozygous and 35% individuals are heterozygous for this null allele in the Caucasian population. Therefore, the interpretation of chitotriosidase activity needs to be in the context of the *CHIT1* genotype.[52,53] Hypomorphic alleles (p.G102S) are also reported to affect the activity of chitotriosidase.[53]

Besides chitotriosidase, ACE and TRAP have been used to monitor patients with Gaucher disease. However, these two biomarkers are not as elevated as chitotriosidase and their use for monitoring has fallen out of favor.

10.4.2 PLASMA PARC/CCL18

The high frequency of chitotriosidase deficiency prompted the search for an alternative marker for Gaucher disease treatment and management. The pulmonary and activation-regulated chemokine (PARC/CCL18) is found to be approximately 30-fold elevated in symptomatic Gaucher patients, which is far more pronounced than ACE and TRAP. Plasma PARC/CCL18 level decreases with ERT, comparably to chitotriosidase. Immunohistochemistry studies have demonstrated that Gaucher cells are the prominent source of PARC/CCL18, making it a surrogate marker for

monitoring therapeutic intervention, particularly for monitoring chitotriosidase-deficient Gaucher patients. However, PARC/CCL18 is less specific for diagnosing Gaucher disease as the level can be massively elevated in other medical conditions associated with inflammation.[54]

10.4.3 PLASMA GLUCOSYLSPHIGOSINE (LYSO-GL1)

Although glucosylceramide (GL1) is the primary lipid storage in Gaucher disease, plasma GL1 is generally not used as a biomarker for monitoring Gaucher disease. Plasma GL1 is only slightly elevated because most of it is present in lipoproteins. The relation between circulating GL1 with Gaucher cell burden in the tissues is also unclear. The deacylated substrate glucosylsphingosine (lyso-GL1) has been found to be markedly increased to approximately 200-fold in plasma of symptomatic non-neuronopathic Gaucher patients and seems to correlate with the current Gaucher cell markers chitotriosidase and PARC/CCL18. Plasma lyso-GL1 level decreases with ERT and a less pronounced reduction with miglustat. Moreover, in vitro study in the cultured macrophages indicated that the elevated circulating lyso-GL1 was originated from Gaucher cells.[55] These findings have been used to justify the use of lyso-GL1 as a biomarker for type 1 Gaucher disease. In a recent larger clinical study, lyso-GL1 has also shown to be correlated with liver and spleen volume. In patients treated with the new SRT drug eliglustat, more reduction of plasma lyso-GL1 was observed compared to ERT-treated patients.[27]

10.4.4 PLASMA (OR URINE) GLOBOTRIAOSYLSPHINGOSINE (LYSO-GB3)

Similarly to Gaucher disease, globotriaosylceramide (Gb3), also named ceramidetrihexoside (CTH), is the primary lipid storage in Fabry disease. However, recently, the deacylated substrate lyso-Gb3 has proven to be the hallmark biochemical marker for Fabry disease manifestation. In vitro exposure with lyso-Gb3, not Gb3, resulted in marked proliferation of smooth muscle cells in culture, which indicates the vasoactive effects of this metabolite.[20] Plasma lyso-Gb3 concentration is elevated 250-fold in classic male Fabry patients, in contrast to the 3-fold increase of Gb3. Plasma lyso-Gb3 level is also unequivocally highly elevated in classic female patients, in contrast to the normal Gb3 level in female carriers. Therefore the plasma lyso-Gb3 can be used as a diagnostic test for Fabry disease in both males and females.[20,56] Additional analogs of lyso-Gb3 have been reported in urine and can be quite abundant in Fabry patients. The diagnostic and monitoring values of these analogs for Fabry disease have yet to be determined.[57–59]

10.4.5 URINE SULFATIDES

Urine sulfatides are extremely low in normal individuals and highly elevated in patients with MLD. This marker is clinically useful in discriminating MLD patients from individuals with pseudodeficiencies. Traditionally, urine sulfatides were

qualitatively visualized by TLC analysis.[60] Quantitative sulfatide analysis using high-performance liquid chromatography (HPLC) revealed a 50-fold increase of urine total sulfatides in MLD patients.[61] Specific and sensitive liquid chromatography and tandem mass spectrometry (LC-MS/MS) analysis have been developed and used for the screening and diagnosis of MLD. A recent study using dried blood spot samples showed 20-fold and 5-fold increases of total sulfatides concentration for early- and late-onset MLD patients, respectively, when compared to normal controls and those with pseudodeficiencies. A corresponding 160-fold and 80-fold increase was found for early- and late-onset MLD in dried urine spot samples suggesting that sulfatides might be a feasible marker for newborn screening for MLD.[62]

10.4.6 OXYSTEROLS

Oxysterols are a group of non-enzymaticoxidative derivatives of cholesterol. Biomarker studies of oxysterols were initiated in patients with NPD-C because of the notion that oxidative stress plays an important role in the pathogenesis of NPD-C disease. Two of the oxysterolscholestane-3β-5α-6β-triol and 7-ketocholesterol were found to be highly elevated in the plasma of NPD-C patients and have been proposed to be the biomarkers for NPD-C.[63,64] These two markers are similarly elevated in patients with NPD-A and NPD-B.[65,66] Moderate elevations of both 3β-5α-6β-triol and 7-ketocholesterol are observed in plasma from patients with cholesteryl ester storage disease. 7-Ketocholesterol is highly elevated in patients with Smith–Lemli–Opitz syndrome. Interestingly, oxysterols are not elevated in patients with familial hypercholesterolemia.[66]

10.5 CONCLUSION

While rare, the sphingolipidoses nonetheless result in significant morbitidy and mortality for those affected individuals. The largest hurdle in recognizing these disorders is considering them on the differential diagnosis. Biomarkers play important roles in screening, diagnosis, and monitoring of sphingolipidoses. Some of these biomarkers are not necessarily the direct substrates of deficient enzymes but may reflect disease pathophysiology. For example, the protein markers chitotriosidase and PARC/CCL18 are not substrates for β-glucosidase but represent the macrophage lipid burden of Gaucher disease. This is also true for oxysterols as biomarkers for NPD-C (as well as NPD-A and NPD-B), which are the results of abnormal cholesterol oxidation that is associated with the pathogenesis of these conditions. These markers are more associated with the disease progression and better for monitoring therapeutic responses. Similarly deacylated disease substrates, lyso-GL1 (Gaucher) and lyso-Gb3 (Fabry) are predicted to be better associated with disease pathology and therefore better biomarkers for Gaucher disease and Fabry disease than their respective disease substrates. These markers are particularly useful in resolving the diagnosis in individuals having pseudodeficiencies or having VOUS or questionable enzyme results.

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