

Thematic Review Series: Genetics of Human Lipid Diseases

Multi-system disorders of glycosphingolipid and ganglioside metabolism

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Abstract Glycosphingolipids (GSLs) and gangliosides are a group of bioactive glycolipids that include cerebrosides, globosides, and gangliosides. These lipids play major roles in signal transduction, cell adhesion, modulating growth factor/hormone receptor, antigen recognition, and protein trafficking. Specific genetic defects in lysosomal hydrolases disrupt normal GSL and ganglioside metabolism leading to their excess accumulation in cellular compartments, particularly in the lysosome, i.e., lysosomal storage diseases (LSDs). The storage diseases of GSLs and gangliosides affect all organ systems, but the central nervous system (CNS) is primarily involved in many. Current treatments can attenuate the visceral disease, but the management of CNS involvement remains an unmet medical need. Early interventions that alter the CNS disease have shown promise in delaying neurologic involvement in several CNS LSDs. Consequently, effective treatment for such devastating inherited diseases requires an understanding of the early developmental and pathological mechanisms of GSL and ganglioside flux (synthesis and degradation) that underlie the CNS diseases. These are the focus of this review.—Xu, Y.-H., S. Barnes, Y. Sun, and G. A. Grabowski. **Multi-system disorders of glycosphingolipid and ganglioside metabolism.** *J. Lipid Res.* 2010. 51: 1643–1675.

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Glycosphingolipids (GSLs) consist of ceramide and one or more attached carbohydrates. The nature of the oligosaccharide head groups categorizes the GSLs into neutral or acidic types due to the absence or presence of sialic acid residues, respectively (Fig. 1). Gangliosides are sialic acid containing GSLs. In eukaryotic cells, GSLs and gangliosides compose 10–20% of the total lipids (1), which are

important to a variety of cellular functions. GSLs and gangliosides are synthesized at the endoplasmic reticulum (ER) and are remodeled during transit from *cis* to *trans* Golgi by a series of glycosyl- and sialyl-transferases. These are then transported to the intracellular compartments and the plasma membrane where they become enriched in microdomains and membrane bilayers. During plasma membrane turnover, GSLs and gangliosides can be internalized and partially or completely degraded in the endosomal/lysosomal system to sphingosine and free fatty acids that are then transported or flipped across late endosomal and lysosomal membranes for recycling or for use as signaling molecules (2, 3).

GSL metabolic pathways

GSL biosynthesis begins with condensation of serine and palmitoyl-CoA catalyzed by serine-palmitoyltransferase (SPT) on the cytoplasmic face of the ER, leading to de novo biosynthesis of ceramide, the core of GSLs (Fig. 1) (3–5). Ceramide consists of a fatty acid acyl chain that var-

Abbreviations: AC, acid ceramidase; ASA, arylsulfatase A; aSMase, acid sphingomyelinase; BDNF, brain-derived neurotrophic factor; CBE, conduritol B epoxide; CERT, ceramide transfer protein; CGT, ceramide UDP-galactosyltransferase; CNS, central nervous system; EET, enzyme enhancement therapy; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; FAPP2, four-phosphate adaptor protein 2; GALC, galactosylceramide- β -galactosidase; Gb3, globotriaosylceramide; GCase, acid β -glucosidase or glucocerebrosidase; GCS, glucosylceramide synthase; GD3 synthase, α -N-acetyl-neuraminidase α -2,8-sialyltransferase; GM3 synthase, LacCer α -2,3-sialyltransferase; GSL, glycosphingolipids; Hex A, β -hexosaminidase A; Hex B, β -hexosaminidase B; IL, interleukin; iNKT, invariant natural killer T; LacCer, lactosylceramide; LacSph, lactosylsphingosine; LSD, lysosomal storage disease; LTP, long-term potentiation; lyso-Gb3, globotriaosylsphingosine; MLD, metachromatic leukodystrophy; NB-DGJ, N-butyldeoxygalactonojirimycin; NB-DNJ, N-butyl-deoxynojirimycin; NGF, nerve growth factor; NPA/B, Niemann-Pick disease Types A and B; nSMase, Neutral sphingomyelinase; Pgp, P-glycoprotein; PNS, peripheral nervous system; Sap, saposin; SPT, serine-palmitoyltransferase; SSIT, substrate synthesis inhibition therapy; TNF α , tumor necrosis factor- α ; UPR, unfolded protein response.

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ies in length and saturation, and a sphingoid base that differs in the number and position of double bonds and hydroxyl groups (6–8). The fatty acid chain length of ceramide is controlled by tissue- and cell-specific ceramide synthases (also called longevity assurance genes) (9). In addition, ceramide can be generated by acid sphingomyelinase (aSMase) hydrolysis of sphingomyelin in the lysosome or at the plasma membrane and by activities of secreted aSMase at the plasma membrane or associated with lipoproteins (Figs. 1 and 2) (10). Neutral sphingomyelinase (nSMase) also cleaves plasma membrane sphingo-

myelin to ceramide (11). In the salvage pathway, lysosomally derived sphingosine can be reacylated (Fig. 2) (12). Once formed, ceramide is sorted to three pathways: 1) GalCer synthesis in the ER that is followed by 3-sulfo-GalCer (sulfatide) synthesis in the Golgi (13, 14); 2) GlcCer synthesis on the cytoplasmic face of the Golgi as the precursor of most GSLs; and 3) ceramide transfer protein (CERT) delivery to the mid-Golgi for sphingomyelin synthesis (15, 16). In the *trans* Golgi lumen, the transfer of a β -galactose onto GlcCer by lactosylceramide (LacCer) synthase forms LacCer (17). Several galactosyl-, *N*-acetylgalactosaminyl-,

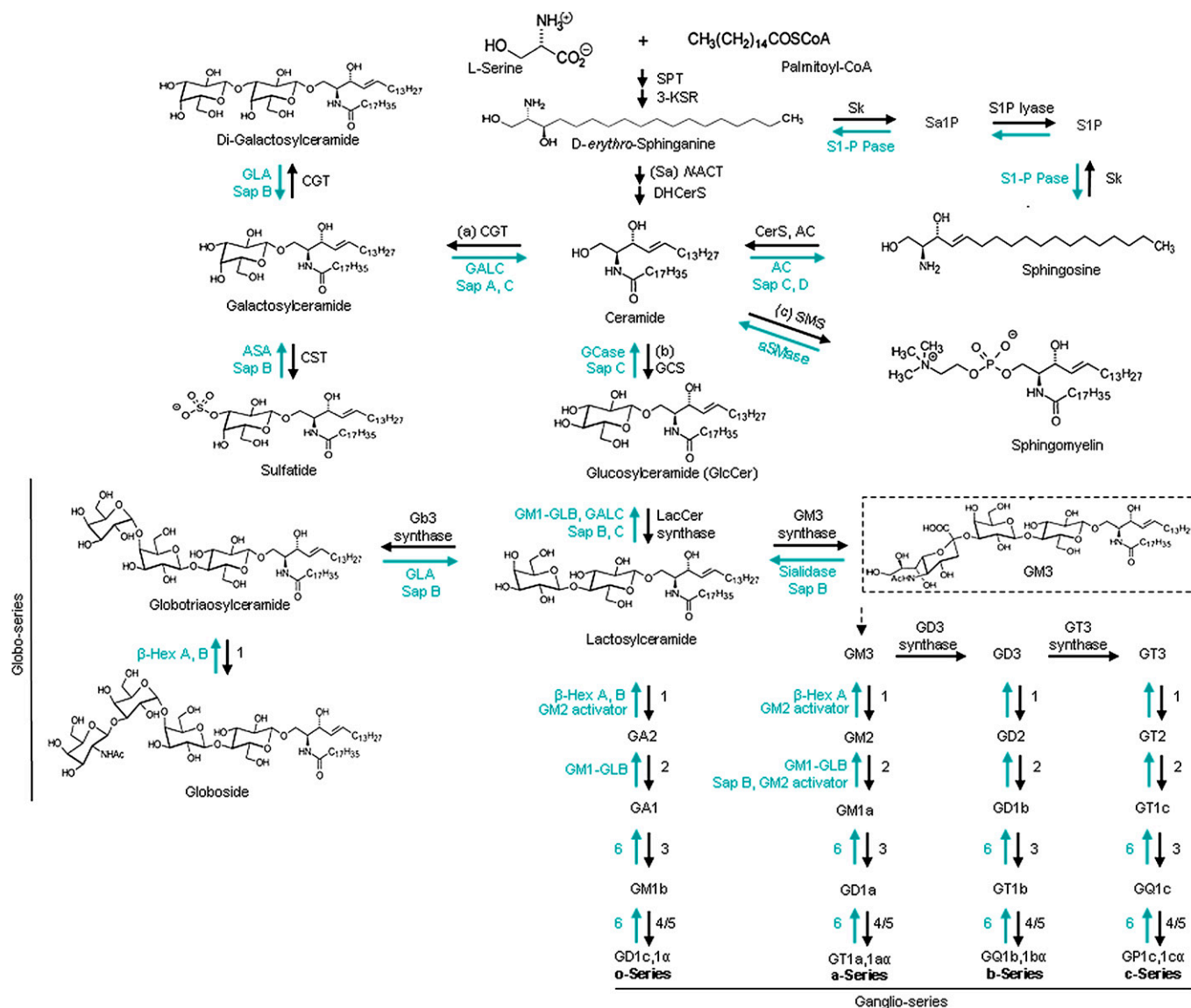


Fig. 1. Schematic view of the GSL metabolism pathways. The synthesis of GSLs and gangliosides progress stepwise and are catalyzed by membranous glycosyltransferases in the ER or Golgi apparatus (see text). The degradation reactions are also sequential and occur within the lysosomes by various hydrolases. The black arrows show the synthesis pathway in the ER and Golgi and the green arrows show the degradation pathway in the lysosome. The enzymes in α -, β -, and γ -ganglioside series are numbered: 1. GM2/GD2/GA2/GT2-synthase (β -1,4-*N*-acetyl-galactosaminyltransferase, GalNAcT), 2. GA1/GM1a/GD1b/GT1c-synthase (UDP-Gal: β -GalNAc β -1,3-galactosyltransferase), 3. sialyltransferase IV, 4. sialyltransferase V, 5. sialyltransferase VII, 6. sialidase. Abbreviations: 3-KSR (3-ketosphinganine reductase), Sk (sphingosine kinase), SaIP (sphinganine 1-phosphate), S1P (sphingosine 1-phosphate), S1-Pase (sphingosine 1-phosphate phosphatase), (Sa) *N*-ACT (sphinganine *N*-acyltransferase), DHCerS (dihydroceramide desaturase), CerS (ceramide synthase, also called longevity assurance genes), SMS (sphingomyelin synthase), GT3 synthase (α -*N*-acetyl-neuraminidase α -2,8- sialyltransferase). The chemical structures are adapted from (3, 36, 100, 313–315) and <http://www.cybercolloids.net/library/sugars/hexoses.php>. Nomenclature of the enzymes and protein are from IUBMB (<http://www.chem.qmul.ac.uk/iubmb/enzyme>) (37, 43, 315).

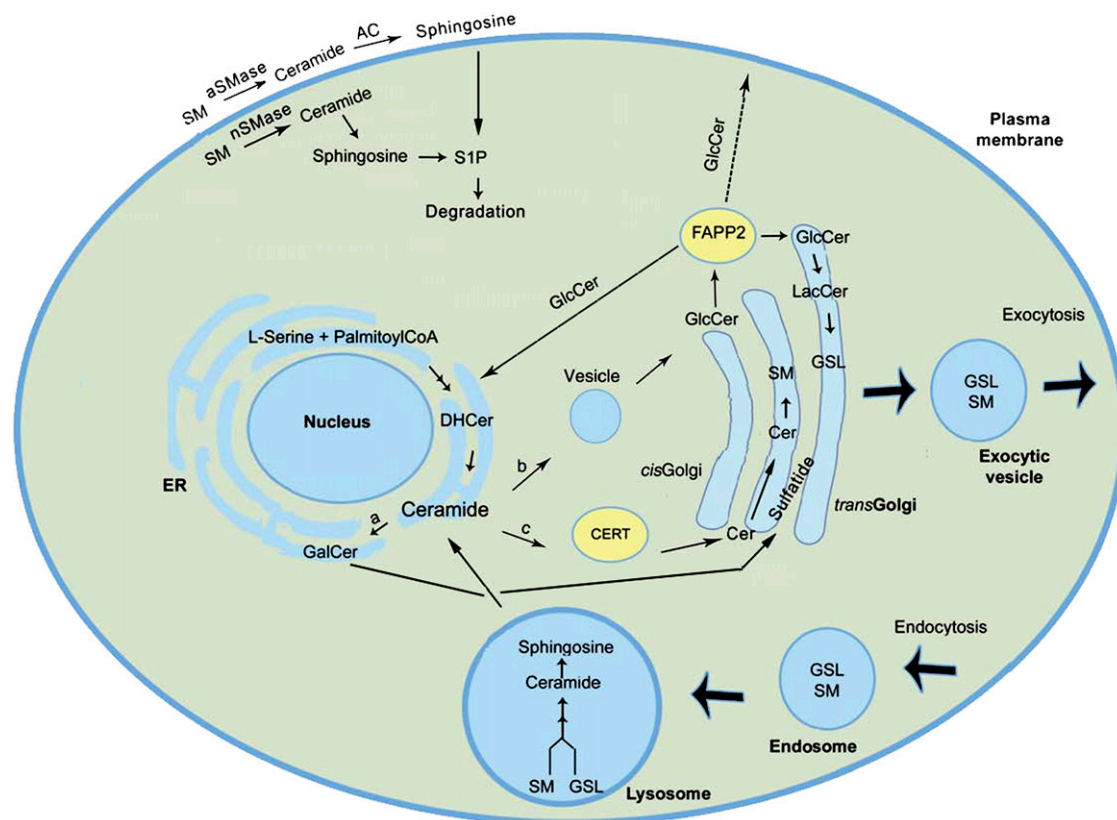


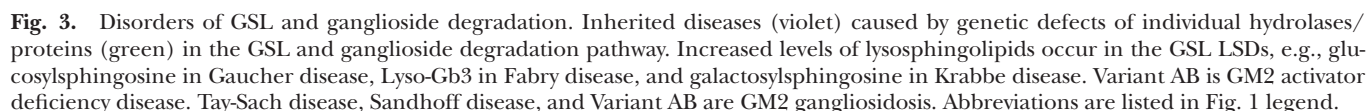
Fig. 2. Intracellular topology of GSL biosynthesis and trafficking. Ceramide, formed by condensation of serine and palmitoyl-CoA on the cytoplasmic face of ER, has one of three fates (16): a) conversion to galactosylceramide (GalCer) in the ER lumen, which is subsequently converted to sulfatide in the mid-Golgi (13, 14), b) vesicular transport to the cytoplasmic face of the cis-Golgi where it is a precursor for GlcCer synthesis (26, 316), and c) transport by ceramide transfer protein (CERT) to the mid-Golgi where sphingomyelin is formed within the lumen (319). Once formed, GlcCer also has several fates (27, 317): 1) transport by FAPP2 to the ER and/or to the *trans*-Golgi lumen where it is converted to lactosylceramide (LacCer) (17, 26, 27), 2) to the cytoplasmic side of the plasma membrane by unknown mechanisms, and 3) to the extracellular matrix by exocytosis. Addition of sialic acid to LacCer initiates synthesis of gangliosides or the sialo-GSL series (318). Ceramide can also be generated through degradation of sphingomyelin in the lysosome or at the plasma membrane by aSMase and nSMase (10). Newly synthesized GSLs can exit the cell by exocytic vesicles while membrane and extracellular GSLs can be transported intracellularly via endocytosis with subsequent degradation sphingosine and free fatty acids by hydrolases in the lysosome (2, 10). Abbreviations: DHCer (dihydroceramide), S1P (sphingosine 1-phosphate), SM (sphingomyelin).

N-acetylglucosaminyl-, and sialyltransferases can elongate the oligosaccharide chain of GSLs along the luminal side of Golgi (18, 19), thereby defining the different series of GSLs. Addition of sialic acid to LacCer forms GM3 ganglioside by LacCer α -2,3-sialyltransferase (GM3 synthase) that initiates synthesis of the ganglioside or sialo-GSL series (Fig. 1). The type of sugars in the oligosaccharide backbone depends on the activity of glycosyltransferases in the Golgi, the cell type, and the developmental or disease stage (19–22) (see below).

In addition to CERT, several other proteins participate in GSL trafficking in the cells. GlcCer can be flipped into the Golgi lumen or across plasma membrane by the ATP-binding cassette transporter [also called multidrug resistance protein, MDR1, or P-glycoprotein (Pgp)]. Although this has been shown for short-chain GlcCer or neutral GlcCer, transport of naturally occurring GlcCer by Pgp has not been shown *in vivo* (23–25). Four-phosphate adaptor protein 2 (FAPP2) can transport newly synthesized GlcCer to the *trans* Golgi and back to the ER (26). It is not clear how FAPP2 transports GlcCer through the cytosol to

the plasma membrane (26, 27). Although glycolipid transfer protein has been shown to have binding affinity for GSLs, transport of GSLs by glycolipid transfer protein has not been reported (28). The mechanisms of intracellular transport of GSLs continue to emerge.

The catabolism of complex GSLs also proceeds by stepwise, sequential removal of sugars by lysosomal exohydrolases to the final common products, sphingosine and fatty acids (Fig. 1). Individual defects in GSL hydrolases (Fig. 3) result in excessive accumulation of specific GSLs in lysosomes leading to the various lysosomal storage diseases (LSDs) (see Table 1). Nonenzymatic proteins are essential to GSL degradation either by presenting lipid substrates to their cognate enzymes or by interacting with their specific enzyme (2). Two genes, *PSAP* (prosaposin) and *GM2A* (GM2 activator protein), encode five such proteins (Fig. 3) (2, 29). Four saposins (A, B, C, and D) or sphingolipid activator proteins (Sap) are derived from proteolytic cleavage of a single precursor protein, prosaposin, in the late endosome and lysosome (30, 31). Each of these saposins has specificity for a particular GSL hydrolase (Table 1).



Many enzymes in GSL metabolic pathways are regulated in response to extra- and intracellular stimuli leading to modulation of bioactive lipid levels (32). The synthesis/degradation pathways (Fig. 1) of GSL metabolism form a network with the product of one enzyme serving as a substrate for other enzymes; e.g., ceramide formed from sphingomyelin may act directly or serve as a substrate for ceramidase, for sphingomyelin synthase, or for GCS, thereby being "converted" to sphingosine, sphingomyelin, and a by product, diacylglycerol, or GlcCer, respectively (33). Also, many cell stimuli modulate the function of more than one of these enzymes in a cell- or tissue-specific manner; upregulation of GCS by tumor necrosis factor α (TNF α) and interleukin (IL)-1 has been reported in the liver (34). The inhibition of GCS and sphingomyelin synthase activities by TNF α was found in the rhabdomyosarcoma cells (35). Thus, the coordinate regulation of such enzymes in response to cellular stimuli alters the lipid flux through this network (Fig. 1) (32). In addition, cellular compartmentalization topologically restricts enzymes and their products to subcellular organelles in which metabolic fluxes are modulated by enzymatic and GSL endocytotic/exocytotic balances (36, 37). Also, disruption of ER- and Golgi-associated endocytotic/exocytotic systems by unrelated pathways (e.g., mucopolysaccharide degrada-

tion) may increase levels of a particular GSL and cause secondary accumulations of other GSLs and gangliosides (38). These secondary storage compounds contribute directly to disease pathogenesis and to complex metabolic events leading to multiple, apparently unrelated substrate storage diseases (See Pathological consequences of disorders in GSL metabolism).

Changes in brain GSL synthesis and metabolism correlate with the stage-specific brain development and function, indicating a coordinated spatial-temporal regulation (**Fig. 4**). Elucidating the timing of GSL synthesis and the alterations of GSL fluxes in the disease states is essential to understanding the pathogenesis and propagation of the various GSL LSDs.

In the mouse embryo, ceramide, the core structure of GSLs, is detected by the 2-4-cell stage following fertilization, before neural tube formation, and is present throughout life (39, 40). The globo-series lipids occur at the 2-cell stage (E0.5) followed by the lacto-series at E1.5, and the ganglio series at E7 (40–42) (Fig. 4). GlcCer is present by E11 during the neuronal stem cell proliferation stage. The concentrations of GlcCer are greater during the neurogenesis and astrocytogenesis at mid-embryonic stages (E12 and E14); these steadily decline at later stages (43, 44). By

TABLE 1. Human and mouse disorders of GSL and ganglioside degradation

Disease (Frequencies)	Gene Symbol, Chr. Location, Name(s)	Species	Gene Defect ^b	Major Storage Materials	Phenotype	Onset	Lifespan	References
Farber disease (lipo granulomatosis) (rare)	<i>ASAHI</i> Chr 8: p22-p21.3 AC, N-acylsphingosine amidohydrolase 1; N-acylsphingosine deacylase, AC, ASAH1	Human	>17 Different mutations including point mutations and splice-site mutations	Ceramide, hydroxyl ceramide	Granulomas, lipid-laden macrophages in the CNS and viscera, mild or no neurological involvement, subcutaneous skin nodules near or over joints	0 days – 1.7 year	3 days–16 year	(96, 108, 321–323)
	<i>Asah1</i> Chr 8: 42425551-42460127(-) <i>Asah1</i> – / –	Mouse	Three copies of targeting constructs (PGK-neo cassette replacing exons 3 through 5) inserted into intron 12	Ceramide	Embryonic lethal	2-cell stage (E0–E1)	<E8.5	(39, 102)
	<i>Asah1</i> Chr 8: 42425551-42460127(-) <i>Asah1</i> +/-	Mouse	Three copies of targeting constructs (PGK-neo cassette replacing exons 3 through 5) inserted into intron 12	Ceramide	Normal, progressive lipid-laden inclusions in liver Kupffer cells.	6 months	Normal lifespan	(102)
NPA (0.5 to 1/100,000)	<i>SMPD1</i> Chr 11: p15.4-p15.1 aSMase, sphingomyelin phosphodiesterase 1, aSMase, ASM, aSMase, Zn-SMase Same as NPA	Human	>100 mutations Single nucleotide deletions, missense mutations	Sphingomyelin, Cholesterol, Bis (monoacylglycerol)phosphate, GlcCer, LacCer Gb3, GM3	Neurovisceral disease, hepatosplenomegaly, failure to thrive, rapid neurodegeneration, foamy cells in multiple organs	3–6 months	2–3 years	(104, 109, 324, 325)
NPB (0.5 to 1/100,000)		Human	A single mutation, a 3-base deletion (ΔR608)	Sphingomyelin, Cholesterol, Bis (monoacylglycerol)phosphate, GlcCer, LacCer, Gb3, GM2, GM3	Visceral disease, minor neurological involvement, mental retardation, foamy cells in multiple organs	Early childhood to adulthood	Adolescence to adulthood	(104, 109, 325)
NPA/B	<i>Smpd1</i> Chr 7: 112702939-112706901 (+) <i>ASM</i> – / –	Mouse	Insertion of a neomycin resistance cassette into exon 3	Sphingomyelin	Hepatosplenomegaly, tremors, ataxia, impaired coordination, lethargic, poor feeding, hunched posture, progressive CNS disease, Purkinje cell loss	2–3 months	4 months	(106, 109)
	<i>Asm</i> <i>Asm</i> – / –	Mouse	Insertion of a neomycin resistance cassette into exon 2	Sphingomyelin, Cholesterol	Dysregulated Ca ²⁺ homeostasis Hepatosplenomegaly, mild tremors, ataxia, lethargic, poor feeding, hunched posture, progressive CNS disease, Purkinje cell loss, atrophy of brain, fertile	2 months	6–8 months	(105)

TABLE 1. Continued.

Disease (Frequencies)	Gene Symbol, Chr. Location, ^a Name(s)	Species	Gene Defect ^b	Major Storage Materials	Phenotype	Onset	Lifespan	References
NPC (1/120,000 to 1/150,000)	<i>NPC1</i> (95% cases) Chr18:q11-q12 NPC1	Human	Infantile and "classic" (severe) phenotype: Premature stop codon mutations, missense mutations in the sterol-sensing domain (SSD), A1054T in the cysteine-rich luminal loop "Variant" (mild) phenotype: Missense mutations (I943M, Y950M, G986S, G992R, P1007A) clustered within the cysteine-rich luminal loop between transmembrane domains 8 and 9	Cholesterol, Sphingomyelin, GlcCer, LacCer and GM2	Progressive Purkinje cell loss, ataxia, dystonia, dementia, variable hepato-splenomegaly, sea blue histiocytes in bone marrow, liver, spleen, and lung	In utero to adult	<6 months to adult	(312, 326–330)
	<i>Npc1</i> Chr 18: 12348202-12394909(-) <i>splm</i> NPC1 – / –	Mouse	Spontaneous mutation domains 8 and 9	Cholesterol, GlcCer, LacCer and GM2	Progressive Purkinje cell loss, demyelination, tremors, hind limb paralysis, poor feeding, foamy macrophages in viscera, hepatosplenomegaly, enlarge lymph nodes, infertile	7 weeks	12–14 weeks	(260, 326, 331)
	<i>Npc1</i> Chr 18: 12348202-12394909(-) <i>npc1th</i> NPC1 – / –	Mouse	Spontaneous mutation, transposon insertion deleting 11/13 transmembrane domains	Cholesterol, Sphingomyelin, lyso(bis)phosphatidic acid, GlcCer, LacCer, GA2, GM2 and GM3	Progressive Purkinje cell loss, demyelination, tremors, hind limb paralysis, poor feeding, foamy macrophages in viscera	5–7 weeks	11–14 weeks	(218, 260, 326, 332–334)
	<i>NPC2/HE1</i> (5% cases) Chr 14: q24.3 HE1, NP-C2, NPC2, HE1/NPC2	Human	Severe disease: 27delG leading to early termination of protein, a missense mutation (S67P), E20X, E118X, S67P, and E20X/27delG mutations Milder disease: splice mutation (VS2+5G→A) in the consensus sequence of the 5' donor site of intron 2	Cholesterol, Sphingomyelin, GlcCer, LacCer and GM2	Same as NPC1	In utero to adult	<6 mos to adult	(326–330)
	<i>Npc2</i> Chr 12: 86097442-86113848(-)	Mouse	Insertion of neo cassette into intron 3	Cholesterol, Sphingomyelin, lyso(bis)phosphatidic acid, GlcCer, LacCer, GA2, GM2 and GM3	Weight loss, tremors, ataxia, generalized locomotor dysfunction	7.9 weeks	13–18.5 weeks	(200)

TABLE 1. Continued.

Disease (Frequencies)	Gene Symbol, Chr. Location, Name(s)	Species	Gene Defect ^b	Major Storage Materials	Phenotype	Onset	Lifespan	References
Krabbe disease (1/100,000)	<i>GALC</i> Chr 14: q31 Galactocerebrosidase β-galactosidase Galactosylceramide β-galactosidase	Human	>60 mutations; Infantile patients: 502C→T polymorphism associated with a 30 kb deletion in intron 10; Late onset patients: missense mutations (R63H, G95S, M101L, G268S, G270D, Y298C, and I234T), nonsense mutation (S7X), a one-base deletion (805delG), mutations interfere with the splicing of intron 1 and intron 6	GalCer, Galactosylsphingosine	Progressive CNS and PNS involvement, hypertonicity/hyperactive reflexes, progressive flaccidity, peripheral neuropathy, severe developmental delay, blindness, spastic paraparesis, dementia and loss of vision in later onset patients, infiltration of characteristic "globoid cells"	3–6 months or 10–40 years	<2 years or adult	(111, 113, 335, 336, 337, 338)
	<i>Galc</i> Chr 12: 99440510-99497547(-) <i>twitcher</i>	Mouse	Spontaneous mutation; G to A transition at codon 339	GalCer, Galactosylsphingosine	Generalized tremors, progressive weakness, wasting, dys/demyelination, abnormal multinucleated "globoid" cells infiltration, gray matter unaffected	3 weeks	6.5–12 weeks	(116, 209, 210, 339, 340)
MLD (1/40,000)	<i>Galc</i> Chr 12: 99440510-99497547(-) Transgenic Krabbe <i>ARSA</i> Chr 22: q13.31- qter Arylsulphatase A cerebrosidase-3-sulfate 3-sulfolysolase, ASA	Mouse Human	Insertion of human mutation (H168C) in exon 5 >400 mutations Deletions, insertions, splice site mutation, and missense mutations	GalCer, Galactosylsphingosine Sulfatide	Tremor, hindleg weakness, microphage infiltration in PNS Difficulty walking after the first year of life, progressive peripheral neuropathies, muscle wasting/weakness, developmental delay, seizures, dementia	25–30 days 6 months–16+ years	58 days 5–63+ years	(120) (122, 123, 341, 342)
	<i>Asa</i> Chr 15: 89302959-89306545(-) <i>Asa</i> -/- <i>Asa</i> + <i>Gal3a1</i>	Mouse Mouse	A neomycin selection cassette inserted into exon 4 ASA -/- mice overexpressing sulfatide synthesizing enzyme CST	Sulfatide Sulfatide	Mild phenotype compared with humans, normal lifespan, no widespread demyelination More severe phenotype of ASA deficiency alone, more aggressive CNS and PNS demyelination	1 year 1 year	Normal lifespan <2 years	(129) (130)
Fabry disease (1/40,000 to 1/117,000)	<i>GLA</i> Chr X: q22-q21 α-Galactosidase A	Human	>400 mutations in the <i>GLA</i> gene, including missense (76.4%), nonsense (16.4%), frameshift (3.6%), and splice site defects (3.6%)	Gb3, lyso-Gb3	Males more severely affected, painful acroparesthesias, angiokeratoma, renal disease, cardiomyopathy, stroke, no clear neuronal involvement, ~50% of heterozygous females affected	Childhood to 30+ years	20–75.4 years	(137, 295, 343–348)
	<i>Gla</i> Chr X: 131122688-131135664(-) <i>Gla</i> -/-	Mouse	A neomycin resistance cassette inserted into fragment containing part of exon 3 and intron 3	Gb3	Normal lipid accumulation in kidneys, endothelium, liver, and peripheral nerve		Normal lifespan	(137, 138, 349)

TABLE 1. Continued.

Disease (Frequencies)	Gene Symbol, Chr. Location, "Name(s)"	Species	Gene Defect ^b	Major Storage Materials	Phenotype	Onset	Lifespan	References
Gaucher disease Type 1 - nonneuropathic (1/800 in Ashkenazi Jewish, 1/100,000 in non-Jewish)	<i>GBA1</i> Chr 1: q21 Acid β -glucosidase, β -glucosylceramide- β -glucosidase, glucosylceramidase, glucocerebrosidase, GCase	Human	Mutation substitution of amino acid asparagine for serine (N370S), N370S/84GG, N370S/L444P associated with Gaucher disease type 1 > 350 mutations associated with all Gaucher disease variants	GlcCer, GM1, GM2, GM3, GD3, Glucosylsphingosine	No neuronal involvement, chronic bone marrow expansion, bony deterioration, hepatosplenomegaly, hypersplenism, hepatic dysfunction, extensive fibrosis and tissue scarring, associated with Parkinson disease	4–25 years	6–80+ years	(95, 141, 226)
	<i>Gba1</i> Chr 3: 89006850-89012603(+)	Mouse	Human disease point mutations (N370S, D409V, D409H, and V394L) at <i>gba1</i> locus in exon 9	GlcCer	Mild phenotype with occasional storage cells in lung, spleen and liver; lipid accumulation in viscera, not in brain	<24 h or 3–7 months	Neonatal lethal (N370S), or normal lifespan (V394L, D409V, D409H)	(350)
		Mouse	Conditional <i>gba</i> knockout in hematopoietic and endothelial cells	GlcCer	Modest storage cells in liver and spleen, progressive splenomegaly, no bone marrow involvement	16 weeks	Normal lifespan	(351)
		Mouse	Conditional <i>gba</i> knockout in hematopoietic endothelial cells	GlcCer	Splenomegaly and microcytic anemia	12 months post induction	Normal lifespan	(352)
Gaucher disease type 2 - neuropathic (1/500,000)	<i>GBA1</i> Chr 1: q21 Acid β -glucosidase, β -glucosylceramide- β -glucosidase, glucosylceramidase, glucocerebrosidase, GCase	Human	A mutation causing leucine to proline change (L444P) associated with the neuronopathic forms of Gaucher disease, numerous other mutations	GlcCer, GM1, GM2, GM3, GD3, Glucosylsphingosine	Progressive CNS and lung involvement, neuronal death/drop-out, visceral involvement as type 1 disease	3 months	<2 years	(95, 141)
	<i>Gba1</i> Chr 3: 89006850-89012603(+)	Mouse	A neomycin resistance cassette was inserted into exons 9 and 10	GlcCer, Glucosylsphingosine	Mice die <24 h, lipid storage in lung, brain, and liver, glucosylsphingosine in brain and viscera		Embryonic lethal	(148)
	<i>Gba</i> - / -	Mouse	Conditional knockout (neo cassette was inserted into intron 8)	GlcCer	Rapid motor dysfunction, severe neurodegeneration, apoptotic cell death in brain, seizures	7 days	14 days	(149)
		Mouse	Conditional knockout (skin rescue), reactivation of a low activity allele in keratinocytes	GlcCer	GlcCer accumulation brain, spleen, and liver; infiltration of Gaucher cells in spleen and liver. Rapid motor dysfunction, severe neurodegeneration, and cell death in CNS	10 days	14 days	(150)
Gaucher disease type 3 -neuropathic (1/100,000)	<i>GBA</i> Chr 1: q21 Acid β -glucosidase, glucosylceramide- β -glucosidase, glucosylceramidase, glucocerebrosidase, GCase	Human	Homozygosity for L444P and D409H, numerous other mutations	GlcCer	Slower progression than Type 2 disease, normal intelligence, short stature with splenomegaly, abnormal eye movements, variable seizures	1–5 years	20–40 years	(95, 141, 353)

TABLE 1. Continued.

Disease (Frequencies)	Gene Symbol, Chr. Location, ^a Name(s)	Species	Gene Defect ^b	Major Storage Materials	Phenotype	Onset	Lifespan	References
Complete Prosaposin/Sap deficiency (rare)	<i>PSAP</i> Chr 10: q21 Prosaposin, Sap precursor, PS, SAP1	Human	1 bp deletion (c.803delG) within the SAP-B domain of the prosaposin gene leads to a frameshift and premature stop codon, A to T transversion in the initiation codon	GlcCer, LacCer, Gb3, Ceramide, Sulfatides, Globotetraosylceramide	Rapid neurological disease, neuronal storage, loss of cortical neurons, astrogliosis, demyelination	3 weeks	16 weeks to 2 years	(176–178, 181)
	<i>Psap</i> Chr 10: 59740375–59765342(+) PS – / –	Mouse	Exon 3 disrupted by insertion of a neomycin selection cassette	GlcCer, LacCer, Gb3, Ceramide, Sulfatides, Globotetraosylceramide	Rapidly neurological disease, hypomyelination, inflammation, multiple GSLs accumulation in various organs	20 days	Neonatal lethality or 5–7 weeks	(190)
	<i>Psap</i> Chr 10: 59740375–59765342(+) PS-NA	Mouse	Expression of transgenic prosaposin in PS – / –	GlcCer, LacCer, Ceramide, Sulfatides,	Ataxia, waddle gait, hypomyelination, inflammation, multiple GSLs accumulation in various organs, Purkinje cell loss	10–12 weeks	7 months	(192)
SapA deficiency late onset Krabbe disease (rare)	<i>PSAP</i> Saposin A, Sap A	Human	A 3 bp deletion in the SapA coding sequence of the prosaposin gene causing deletion of a conserved valine at amino acid number 11 of the SapA protein	GalCer, Galac tosylsphingosine	Normal development, rapid neurological deterioration, loss of acquired milestones, vegetative state, eye contact and spontaneous movement at end stage	3.5 months	8 months	(187)
	<i>Psap</i> Sap A – / –	Mouse	Amino acid substitution in the SapA domain on exon 4, Cys → Phe	GalCer, Galac tosylsphingosine	Hind limb paralysis, tremors, shaking at end stage, pathology and biochemistry identical but milder than <i>twit</i> mice	2.5 months	5 months	(191)
SapB deficiency - variant MLD (rare)	<i>PSAP</i> Sap B	Human	Mutations destroy glycosylation sites, in-frame deletion of the first 21 bases of exon 6	Sulfatide, Gangliosides, LacCer, Gb3, Digalactosylceramide	Variant form of MLD, normal ASA activity in white blood cells, increased lipid in urine	1–10 years	5–22 years	(124)
	<i>Psap</i> Sap B – / –	Mouse	Amino acid substitution was introduced into the cysteine in SapB domain on exon 7, Cys → Phe	Hydroxy and nonhydroxy fatty acid sulfate, LacCer, Gb3	Neuromotor deterioration, minor head tremor; closely resembles MLD mice, no demyelination unlike human disease	15 months	<2 years	(194)
SapC deficiency (rare)	<i>PSAP</i> Sap C	Human	Neuronopathic: mutations on SapC domain (p.C382G, p.C382F, p.C315S) and premature stop codon in the SapD domain (p.Q430X), each on separate allele	GlcCer	Hepatosplenomegaly and severe mental deterioration, no Gaucher cells in bone marrow, slight retardation, focal seizures then generalized, ataxia, tremors	1–8 years	14–15.5 years	(124, 188, 189, 354–356)
			Non-neuronopathic: missense mutation on the SapC domain and the initiation codon mutation (p.L349P/p.M1L)		opthalmoplegia, dysarthria, spastic tetraparesis, normal GCase activity			

TABLE 1. Continued.

Disease (Frequencies)	Gene Symbol, Chr. Location, ^a Name(s)	Species	Gene Defect ^b	Major Storage Materials	Phenotype	Onset	Lifespan	References
	<i>P3ap</i> Sap C-/-	Mouse	Amino acid substitution on the SapC domain in exon 11, Cys → Pro	GlcCer, LacCer, LacSph	Progressive ataxia, Purkinje cell loss, cerebellum atrophy, impaired hippocampal LTP, slower disease progression than human, reduced GCase activity	1 year	<2 years	(193)
	<i>P3ap</i> Sap D-/-	Mouse	Amino acid substitution on the SapD domain in exon 13, Cys → Ser	Ceramide and hydroxyl ceramide	Renal degeneration, progressive polyuria, progressive, selective loss of cerebellar Purkinje cells	6 months	15 months	(98)
	<i>P3ap</i> Sap CD-/-	Mouse	Amino acid substitutions were introduced into cysteine on SapC, (Cys → Pro) and D domains (Cys → Ser)	GlcCer and α-hydroxy ceramides	Severe neurological phenotype with ataxia, kyphotic posturing, hind limb paralysis, lipid accumulation in brain and kidney	4 weeks	8 weeks	(266)
β-glucosidase (<i>Gba</i>) + prosaposin/Sap deficiency	<i>Gba</i> + <i>P3ap</i> V394L/PS-NA D409H/PS-NA	Mouse	<i>Gba</i> point mutants (V394L or D409H) crossed with mice expressing a low level <i>P3ap</i> transgene (NA)	GlcCer, LacCer, Gb3	Engorged macrophages infiltration in liver, lung, spleen, thymus, Purkinje cell loss, ataxia	12 weeks	22 weeks	(357)
	<i>Gba</i> + <i>P3ap</i> V394L/Sap C-/-	Mouse	<i>Gba</i> point mutant crossed with SapC-deficiency mice	GlcCer, glucosylsphingosine	Hind limb paresis, axonal degeneration in CNS, hippocampal LTP attenuated	30 days	48 days	(205)
GM1 gangliosidosis (1/100,000 to 1/300,000)	<i>GLB1</i> Chr 3: p2.33 GM1-β-Galactosidase acid-β-galactosidase	Human	78 missense/nonsense mutations, 10 splicing mutations, 7 insertions, and 7 deletions Missense/nonsense mutations are primarily located in exon 2, 6, and 15	GM1, GA1, GM2, GM3, GD1A, lyso-GM1, GlcCer, LacCer, oligosaccharides, keratan sulfate	Hepatosplenomegaly, localized skeletal involvement, CNS deterioration, seizures, mental regression, dystonia, gait disturbances, dysarthria	Birth to 3 years	1–30 years	(155, 358, 359)
	<i>GLB1</i> Chr 9: 114310237-114383495(+) GM1 gangliosidosis mouse	Mouse	A neomycin resistance gene was inserted into exon 6	GM1, GA1, GM2, GM3, GD1A, lyso-GM1, GlcCer, LacCer, oligosaccharides, keratan sulfate	Progressive spastic diplegia, clinical, pathological, biochemical manifestations similar to human	4 months	7–10 months	(156, 360)
GM2 gangliosidosis Tay-Sachs (variant B) (1/4000 in Jewish 1/320,000 in non-Jewish)	<i>HEXA</i> Chr 15: q23-q24 β-hexosaminidase A (αβ) β-hexosaminidase S (αα) Hexosaminidase A	Human	Single nucleotide changes, deletions or insertions of varying size	GM2, GD1aGalNAc, GA2, lyso-GM2	CNS disease, rapid mental and motor deterioration, variable early dementia in adults	3 months to adult	2–40 years	(161, 162)
	<i>Hex A</i> Chr 9: 59387504-59412914(+) Tay-Sachs mouse	Mouse	A neomycin resistance cassette was inserted into and disrupted exon 8 of the gene	GM2, GD1aGalNAc, GA2, lyso-GM2	Mouse normal, rare storage neurons in posterior horn of spinal cord, no obvious storage cells in viscera		Normal	(361, 362)

TABLE 1. Continued.

Disease (Frequencies)	Gene Symbol, Chr. Location, ^a Name(s)	Species	Gene Defect ^b	Major Storage Materials	Phenotype	Onset	Lifespan	References
GM2 gangliosidosis (variant 0) (1/1000,000 in Jewish non-Jewish)	<i>HEXB</i> Chr 5: q13 β-hexosaminidase A (αβ) and B (ββ) Hexosaminidase A/B, HexA/HexB	Human	The most common mutation is a deletion of 16 kb including the <i>HEXB</i> promoter, exons 1–5, and part of intron 5	GM2, GD1aGalNAc, globoside, oligosaccharides, lyso-GM2	Neurologic symptoms begin within first year, facial dysmorphism, skeletal dysplasia, hepatosplenomegaly	5–6 months	1.5–5.5 years	(161, 172, 363)
GM2 gangliosidosis (rare)	<i>Hexb</i> Chr 13: 97946362-97968225(-) Sandhoff mouse	Mouse	A neomycin resistance cassette was inserted into and disrupted exon 13 of the gene	GM2, GD1aGalNAc, globoside, oligosaccharides, lyso-GM2 (PND2)	Motor function deterioration, head tremor, ataxia, bradykinesia, impaired balance, hind limb paralysis	3 months	5 months	(169)
GM2 gangliosidosis (rare)	<i>GM2A</i> Chr 5: q31.3-q33.1 GM2 Activator GM2 ganglioside activator protein	Human	Single nonsense mutation in exon 2	GM2, GA2 (minor)	Infantile acute encephalopathic phenotype, closely resembles Tay-Sachs disease	1 month	5 months–5 years	(165, 172, 364)
GM2 Activator deficiency (Tay Sachs variant AB), (rare)	<i>Gm2a</i> Chr 11: 54911617-54924400(+) Gm2a-/-	Mouse	A neomycin resistance cassette replaced exon 3 and exon 4	GM2, GA2(minor)	Mouse normal, minor storage in cerebellum unlike Tay-Sachs disease mice		Normal	(175)

^a Mouse Genome Database at the Mouse Genome Informatics Web site, The Jackson Laboratory, Bar Harbor, ME. World Wide Web (URL: <http://www.informatics.jax.org>), January, 2010.

^b Detailed mutation information for each disease can be found in the references.

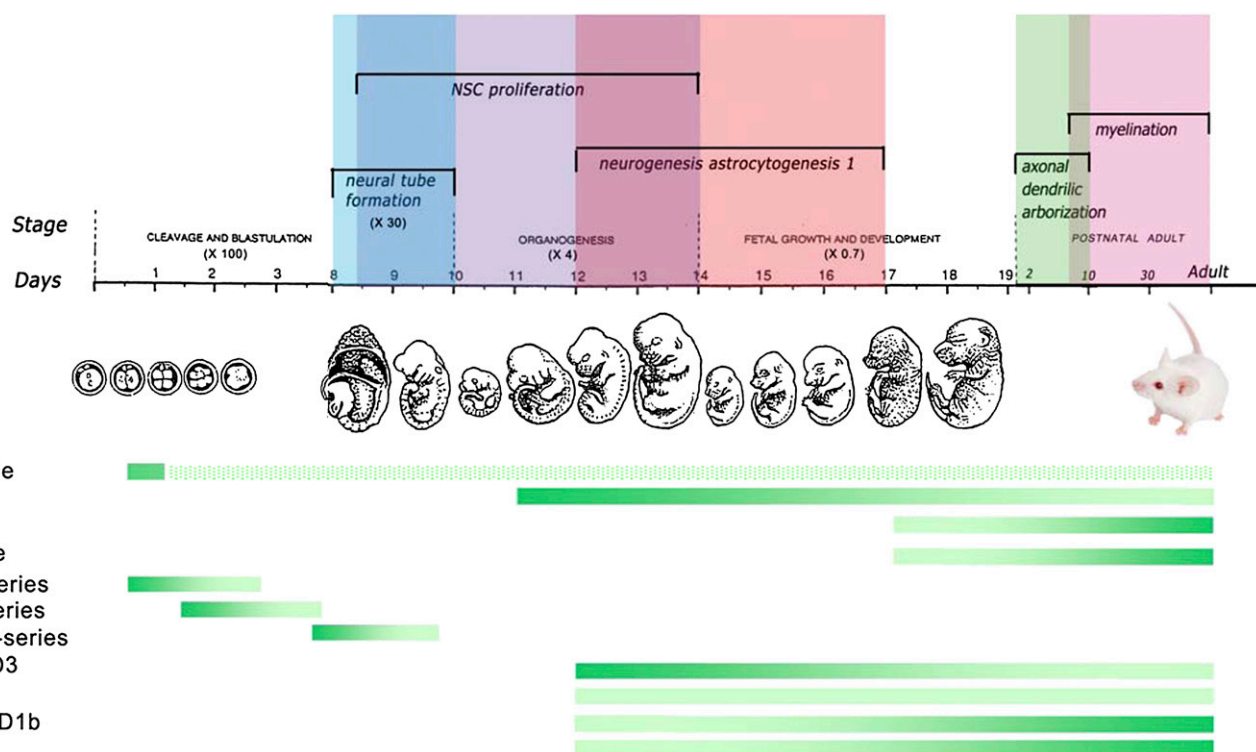


Fig. 4. GSLs and gangliosides in developmental stages of the nervous system. The diagram shows GSL profiles during development of the mouse nervous system. Mouse embryonic developmental stages are from single cell (~ day 1), 16 cells (~ day 3) to fetal (day 19). The mouse embryonic and neural developmental stages are described (320). Colored (vertical) bars above the timeline illustrate overlapping developmental stages. The horizontal gradient bars show the dynamic changes of GSL and ganglioside levels that occur during the stages of mouse embryo and neural development based upon experimental observations (22, 39, 40, 43, 44) (Sun et al., unpublished observations). The dark green indicates increased levels of the specific lipids and the less dense green implies lower levels. Ceramide could be identified at 2-4 cell stage (39). The hatched bar demonstrates the hypothetical ceramide levels since ceramide synthesis remains active throughout life.

postnatal day 11, GlcCer continues to decrease coincidentally with axonal dendritic arborization during adulthood (43) (Sun et al., unpublished observations). Around E16 during fetal development, the central nervous system (CNS) ganglioside distribution patterns shift sequentially from predominantly GM3 and GD3 to the a- and b-series (GD1a, GD1b, and GT1b) and GM1 (22). GalCer and sulfatide become detectable at E17 and predominate at maturity in the adult mouse brain (43). A steady increase in sulfatide content begins at about postnatal day 7 and continues into adulthood in the rat, correlating with active myelin formation. This is regional, because sulfatide levels in the spinal cord are 5-fold higher than in cerebral cortex (45).

These lipid shifts are primarily modulated by differential expression of specific glycosyltransferases because glycosidases vary little during brain development. GD3-synthase and GM2/GD2-synthase are key enzymes in ganglioside biosynthetic pathways (Fig. 1). GM2/GD2-synthase expression significantly increases during in utero development (46–48) and ex vivo in differentiating primary neural precursor cells (43). In comparison, the key enzymes, GCS, ceramide UDP-galactosyltransferase (CGT), and GM3 synthase are not differentially expressed during development (43).

Defects of GSL biosynthesis in mice and some human patients provided insights into the importance of GSLs and gangliosides during brain development (Table 2).

The specific roles of GSLs in survival, proliferation, and differentiation have been demonstrated by knockout mice with embryonic lethality (GCS and SPT) (49, 50) and severe neurodegenerative diseases (CGT) (51, 52). Disrupted ganglioside synthesis in mice with either GM3 synthase (53, 54), GM2/GD2 synthase (55), or α -N-acetylneuraminidase α -2,8-sialyltransferase (GD3 synthase) (56) knocked out causes differential neurological impairments with normal brain formation. Mice lacking CGT are unable to synthesize galactosylceramide. They showed normal growth but had nerve conduction deficits (51, 52). A compensatory increase in GlcCer was observed. The early embryonic lethality of the GCS-deficient mouse delineates the essential roles of complex GSLs in survival (50). Furthermore, the neurodegeneration in the GCS neuronal knockout showed the importance of GSLs in the maintenance of the CNS (57, 58).

To date, three human diseases are associated with mutations in enzymes involved in de novo GSL synthesis (59–61). SPT consists of three subunits and catalyzes the first step in sphingolipid synthesis (Fig. 1). Six missense mutations in SPT long-chain subunit 1 (*SPTLC1*) were reported in 26 families that have autosomal dominant hereditary sensory and autonomic neuropathy type 1 (62, 63). These SPT mutations caused changes in substrate specificity that led to formation of two atypical neurotoxic

TABLE 2. Disorders of GSL and ganglioside synthesis

Affected Gene(s)	Enzyme, Name(s)	Affected GSLs	Species	Phenotype	Lifespan	References
<i>SPTLC1</i>	Serine palmitoyltransferase, long chain base unit 1, SPTLC1	Increased de novo glucosyl ceramide, accumulation of neurotoxic 1-deoxy-sphinganine and 1-deoxymethyl-sphingonine	Human	Distal sensory loss, sensorineural hearing loss starting between 20 and 50 years	Normal	(59, 60, 62)
<i>Sptlc1</i> <i>Sptlc2</i>	Serine palmitoyltransferase, long chain base unit 1 Sptlc1 or 2, SPT Sptlc1 ^{-/-} and Sptlc2 ^{-/-} Sptlc1 ^{+/-} and Sptlc2 ^{+/-}		Mouse	Embryonic lethal		(49)
		Decrease ceramide and sphingosine in liver and plasma, decreased SIP levels in plasma	Mouse	Altered homeostasis	Normal	(49)
<i>SIAT9</i>	GM3 synthase CMP-NeuAc:LacCer α 2,3-sialyltransferase LacCer α 2,3-sialyltransferase	No a- and b-series gangliosides	Human	Developed severe epileptic seizures starting between 2 weeks and 3 months	2–18+ years	(64)
<i>Siat9</i>	GM3 synthase ST3 β -galactoside α 2,3-sialyltransferase 5		Mouse	Hearing loss detected 13 days after birth; increased insulin sensitivity detected in 6–8 week old mice	Normal lifespan	(53, 54)
<i>Siat8a</i>	GD3 synthase CMPsialic acid:GM3 -2,8-sialyltransferase α -N-acetyl-neuraminate α -2,8-sialyltransferase	No b-series ganglioside	Mouse	Normal	Normal lifespan	(56)
<i>GALGT1</i>	GM2/GD2 synthase UDP-N-acetyl-D-galactosamine: UDP-GalNAc: GM3 N-acetylgalactosaminyltransferase	GM3 accumulation	Human	Poor physical and motor development, frequent seizures	2–3 months	(61)
<i>Galgt1</i>	GM2/GD2 synthase UDP-N-acetyl-D-galactosamine: GM3/GM2/GD2 synthase, β -1,4-N-acetyl-galactosaminyltransferase, GalNAcT.	Lack all complex gangliosides, express high levels of GD3 and GM3	Mouse	Slight reduced neural conduction velocity due to axonal and degeneration and demyelination in CNS and PNS, detected in 10–16 week old mice, males infertile	> 1 year	(55, 365, 366)
<i>Galgt1</i> + <i>siat8a</i>	GM2/GD2, and GD3 synthases	GM3 ganglioside only	Mouse	Fatal audiogenic seizures and peripheral nerve degeneration detected in 2–4 month old mice, males infertile	3–9 mos	(367)
<i>Galgt1</i> + <i>Siat9</i>	GM2 and GM3 synthases	No higher order gangliosides	Mouse	Severe neurodegeneration hind limb weakness, ataxia, and tremors starting at 2 weeks old	2–5 mos	(368)
<i>Cgt</i>	Galactosyltransferase CGT	Lack galactosylceramide and sulfatide	Mouse	Severe generalized tremor and mild ataxia starting between 1 and 2 weeks	2.5–4 weeks	(52)
		Lack galactosylceramide and sulfatide	Mouse	Severe dys/demyelination and loss of nerve conduction velocity starting between 1 and 2 weeks	3.5–4 weeks	(51)
<i>Ugcg</i>	Glucosylceramide synthase GCS	No GlcCer-based gangliosides	Mouse	Embryonic lethal	E7.5	(50)
	<i>Ugcg</i> knockout in neurons and glial cells	Trace levels of GlcCer-based gangliosides in neural and glial cells	Mouse	Cerebellum and peripheral nerve dysfunction, reduced axon branching of Purkinje cells beginning at 5 days after birth	3 weeks	(58)
	<i>Ugcg</i> knockout in keratinocyte cells	Decreased GlcCer in epidermis	Mouse	Desquamation of the stratum corneum, transdermal water loss leading to death starting from 3 days postnatally	5 days	(369)

deoxyl-sphingoid bases by condensation of palmitoyl-CoA and alanine or glycine, instead of serine (62). Thus, hereditary sensory and autonomic neuropathy type 1 is caused by a gain-of-function mutation. GM3 synthase catalyzes the first step of complex gangliosides synthesis (Fig. 1). A loss-of-function mutation in GM3 synthase (*SIAT9*) was identified in a cohort characterized by autosomal recessive infantile-onset symptomatic epilepsy syndrome (64). A nonsense mutation in exon 8 of the GM3 synthase gene produced premature termination that could abolish enzymatic activity (64). Affected individuals are homozygous for the mutation while heterozygous carriers are unaffected (64). A single case of GM2 synthase deficiency was reported by biochemical characterization only (61). The patient died at 3 months after presenting with abnormal motor function and seizures. GM3 accumulated in the brain and liver (61).

Phenotypic analyses from GSL synthase-deficient mice reveal that lack of all GSL is incompatible with embryonic development (49, 50). Simple to complex GSLs are nonessential for early brain development but are important for brain maturation and maintenance (51–56). Compensatory interchange between various GSLs also signifies additional complexities in their metabolism.

Distribution and functions of GSLs

The sialic acid-containing GSLs are ubiquitously expressed in the outer leaflet of the plasma membranes, e.g., liver (65), and are most abundant in CNS and other nervous tissues (22, 65, 66), but there is significant tissue/regional variability in distribution. High concentrations of GD1a are present in extraneural tissues, erythrocytes, buffy coat, bone marrow, testis, spleen, and liver, whereas different GSLs are in high amounts in other tissues, e.g., GM4 in kidney, GM2 in bone marrow, GM1 in erythrocytes, and GM3 in intestine (19). GA2, GM2, and GM3 are at very low or nondetectable levels in normal brains from humans, cats, and mice (66). Cellular specificity is exemplified by GD1b localization to small neurons, whereas O-Ac-disialoganglioside localizes to large neurons (67). In liver, GM1 was detected on the canalicular and sinusoidal lining cells, but nearly absent from liver parenchymal cells; GM1 was increased in hepatic sinusoidal membranes during cholestasis (68). In skeletal muscle, the neutral GSLs and gangliosides were mostly in membrane vesicles with GlcCer predominating and only trace levels of LacCer (69, 70). GalCer, sulfatide, and sphingomyelin are structural to myelin sheaths and are major lipids in oligodendrocytes and Schwann cells (71). In skin, ceramide comprises about 50% of total epidermal lipid and is generated from GlcCer in the lamellar bodies (72). These cellular microdifferences in GSLs indicate subtle, yet potentially important, metabolic roles that have not been elucidated. Cellular distribution of GSLs can be altered in pathologic states. In MDCK kidney cells, mitochondrial and peroxisomal GSLs are very low/absent (73), whereas GD1b and GD3 had high contents in mitochondria from malignant hepatomas (74). During the apoptotic process, GD3 is rapidly synthesized and relocated from the *cis* Golgi to mitochondria, leading to the opening of mitochondrial

permeability transition pores and release of apoptogenic factors (75).

GSLs have differentially ordered domains to create selectivity in membrane transport that is important in the spatial organization of cells (76). GSLs are enriched to 30–40 mol% in some cell types, e.g., in the apical membranes of intestinal and urinary tract epithelial cells or in the myelin of axons (77–79). Complex GSLs are generally enriched on the plasma membrane, but GlcCer is mostly localized to intracellular membranes (80). GSLs are found in the vacuoles of the exocytotic and endocytotic systems with low content in the ER (65, 73). Complex GSLs are not translocatable to the cytosolic surface of Golgi (81).

Rafts are ordered structures that participate in cell recognition and signaling. These microdomains or lipid rafts are composed of GSLs, sphingomyelin, and cholesterol (33, 82) that provide environments for enrichment of specific proteins (83) in a variety of membranes. Examples include Src family kinases that are important for signal transduction (84), glycosylation-dependent adhesion/recognition and signaling (85), and G-protein-coupled receptor-signaling (86).

Additional roles of GSLs, including ceramide, sphingosine, sphingosine-1-phosphate, lysoglycolipids, and lysogangliosides, are their inhibition or activation of apoptosis, proliferation, and stress responses (87). Cellular ceramide is an important second messenger in signal transduction that modulates a variety of these physiologic and stress responses (Fig. 2) (87). Several possible intracellular ceramide-target enzymes and signaling pathways have been proposed, including the activation of Ras/extracellular signal-regulated kinase (ERK)-MAPK cascade (88), protein kinases PKC ζ (89), kinase suppressor of Ras/MAPK (90), SAPK/JNK signaling pathway (91), Raf-1 (92), protein phosphatases PP1 and PP2A (93), and JNK (91); all contribute to the control of cell growth, proliferation, and death through various downstream intermediates.

For the field of GSL LSDs, a major challenge remains in the unification of the cell and developmental specificity, biological functions, and pathogenic mechanisms. Given the plethora of diverse and essential cellular functions, the severity of disruptions in GSL metabolism would be anticipated to include more than just accumulation of the GSL in cells and architectural distortions of specific cell types. Such mechanistic understanding of the GSL storage diseases is just beginning to be delineated (See below).

DISORDERS OF GSL AND GANGLIOSIDE DEGRADATION IN HUMANS AND MOUSE MODELS

The catabolic defects in neutral GSL and ganglioside metabolism result in LSDs with complex, multi-system progressive diseases, including neurodegeneration, that can present early in utero or childhood. All the catabolic enzymes for these diseases exist in the lysosomes (Fig. 3). Later onset and more attenuated variants of each disorder have been or are anticipated to be present in humans (Table 1). All these

diseases are autosomal recessive except Fabry disease, which is X-linked. The phenotypic variants and the molecular causes have been variably delineated. Attempts have been made to correlate the genotype with phenotype. This is a complex and incomplete task because of the variability in presentation, nonuniform clinical delineation, and lack of complete allele characterization. However, the residual in situ hydrolase activities in patients are a major, but not unique, determinant of the age at onset and/or severity of the disease. Work by Sandhoff et al. (94) clearly established in situ residual activity as a critical correlate of phenotype with a direct, albeit nonlinear, relationship between this activity and the age at onset in Tay-Sachs disease and metachromatic leukodystrophy (MLD). Extensive clinical data show similar correlations in Gaucher disease type 1 (95). Mouse models of several LSDs have been developed (Table 1). Although the mouse phenotypes are qualitatively similar to its corresponding human disease, such models can show substantial differences because of differences in GSL metabolism between humans and mice. Analyses of such mouse models of GSL synthesis and degradation have provided insight into early pathological mechanisms of GSL and ganglioside flux aberrations.

The disorders of ceramide, GSLs, and gangliosides degradation in humans and mice are summarized below. The phenotypes and genotypes of these disorders are summarized in Table 1.

Disorders of ceramide degradation

Disorders of ceramide degradation are caused by acid ceramidase (AC; EC 3.5.1.23) deficiencies that lead to Farber disease, a.k.a. Farber lipogranulomatosis (96, 97). AC catalyzes the hydrolysis of ceramide to free fatty acid and sphingosine, and its function is optimized by saposin D (Figs. 1 and 3) (98). However, AC can also synthesize ceramide from sphingosine and free fatty acids (Fig. 1) (99, 100). This reverse reaction is optimally catalyzed at pH ~6.0 compared with the hydrolytic activity optimum of pH ~4.5, suggesting that the two reactions likely occur in different subcellular compartments. There are seven variant forms of Farber disease that are distinguished by severity and tissue involvement (96). Although allogeneic hematopoietic stem cell transplantation in Farber disease patients without CNS manifestation showed improvement in joint movement (101), there is currently no effective therapy for this disease. The AC-deficient mouse (*Asah1*^{-/-}) undergoes apoptotic death at the 2-cell stage (E1; Fig. 4) because of excessive ceramide accumulation (39, 102), demonstrating that AC is expressed in the embryo and that ceramide degradation is essential for survival beyond the 2-cell stage (E1) (102). In addition to increasing ceramide pools, loss of AC activity is thought to concurrently reduce pools of sphingosine and sphingosine-1-phosphate, two lipids known to promote cell growth and differentiation (103).

Disorders of GSL degradation

Mutations in the *SMPD1* gene that encodes aSMase (ASM; EC 3.1.4.12) cause two types of Niemann-Pick dis-

ease: Types A and B (NPA and B) (Fig. 1, Table 1). NPA presents a neurovisceral phenotype, whereas NPB exhibits visceral manifestation (104). The ASM-deficient mice, developed independently in two laboratories, resemble NPA or NPB phenotypes (Table 1) (105, 106). Neither model has detectable lysosomal ASM activity in any tissue but has reduced levels of plasma membrane-bound nSMase activity, which cleaves nonlysosomal sphingomyelin at neutral pH (Fig. 2) (106, 107). The ASM gene is expressed prior to E11 during neuronal stem cell proliferation, and deficient ASM activity, due to genetic defects, can lead to loss of signaling molecules that regulate their proliferation very early on in brain development (108, 109).

Krabbe disease, globoid leukodystrophy disease, is a rapidly progressive, demyelinating disease that results from insufficient cleavage of galactosylceramide and galactosylsphingosine by galactosylceramide- β -galactosidase (GALC; EC 3.2.1.46) (Table 1, Fig. 3). This enzyme has specificity for both galactosylceramide and galactosylsphingosine, the latter being a specific substrate for this enzyme whereas galactosylceramide can be cleaved by at least two other lysosomal β -galactosidases (Fig. 1) (110), potentially accounting for the lack of large accumulations of galactosylceramide in the CNS (111). GALC has a number of nonsynonymous polymorphisms (missense or nonsense mutations) that affect the expression and function of the enzyme (111, 112). There are four forms of the disease that differ in age of onset (113). Secondary pathological changes including reactive astrocytic gliosis, infiltration of unique and often multinucleated macrophages (globoid cells), and other inflammatory responses accelerate disease progression (111). Bone marrow/stem cell transplantation with histocompatible cells has shown some positive effects on the CNS and peripheral nervous system (PNS) phenotypes in the infantile onset disease when performed prior to the onset of significant neurological involvement. However, the dementia and other aspects of the CNS and PNS disease eventually lead to death (114). GALC-deficient mice (*twitcher* mouse) were discovered at the Jackson Laboratory in 1976 (115, 116). This naturally occurring model is an excellent model of the infantile human disease (117–119). A transgenic globoid leukodystrophy disease Krabbe disease model was created by ‘knock-in’ of a missense mutation (H168C) (120). The analyses of these models demonstrated that there is a correlation between accumulation of galactosylsphingosine and the neuronal defects. The accumulation of galactosylsphingosine was detected before myelin formation (119). Bone marrow transplantation in *twitcher* mice stabilized galactosylsphingosine at low levels accompanied with remyelination (121). These findings provide support for galactosylsphingosine as the offending agent responsible for triggering proinflammation, oligodendrocyte depletion, and dysmyelination in Krabbe disease.

MLD is caused by a deficiency of arylsulfatase A (ASA; EC 3.1.6.8) that leads to the accumulation of sulfatide, a major lipid of myelin, in the CNS and PNS (Fig. 3) (122, 123). Currently, five allelic forms of ASA deficiency exist (Table 1). Also, there are two nonallelic variants of ASA

deficiency: 1) MLD due to saposin B deficiency (described below) leads to inability to cleave sulfatide and globotriaosylceramide (Gb3) in vivo as a result of the activator protein deficiency and impaired lipid presentation to the enzyme, even though ASA and α -galactosidase A are normally expressed and without mutations (124); and 2) multiple sulfatase deficiency is a disorder that combines features of a mucopolysaccharidosis with those of MLD (125) and results in a deficiency of all cellular sulfatases due to defects in a posttranslational enzyme essential for modification of an active site cysteine that is present in all sulfatases (122, 126). Details of multiple sulfatase deficiency have been reviewed (125, 127). For the infantile and juvenile MLD variants, bone marrow/stem cell transplantation has been attempted with initial improvement/stabilization followed by later progression of the CNS and PNS diseases (128). ASA-deficient mice (*Asa*^{-/-}) have a very slowly progressive sulfatide storage resembling the early stages of human cases (129). A more severe phenotype of ASA deficiency was achieved by overexpressing the transgene for the sulfatide-synthesizing enzyme, galactose-3-O-sulfotransferase (CST), encoded by *Gal3st1*, in oligodendrocytes (130). These models have been used in preclinical trials of MLD (131, 132).

Fabry disease, the only X-linked GSL storage disease, is due to a deficiency of α -galactosidase A (133), the enzyme that cleaves Gb3 and di-galactosylceramide (Fig. 3, Table 1). Uncharacteristic of most X-linked traits, both males and females have significant to major clinical involvement (134). Affected patients accumulate the substrate, Gb3, in most tissues and organs (135), but the progressive deposition of Gb3 in capillary endothelial cells leads to many of the morbid manifestations (136). Increased deacylated Gb3, globotriaosylsphingosine (lyso-Gb3), in patient plasma suggests that it may be involved in pathogenesis of Fabry disease (137). The mouse model of Fabry disease (*Gla*^{-/-}) has accumulation of Gb3 in peripheral nerves and alterations of sensory nerve function that resembles neuropathic pain in Fabry disease patients (138). Gb3 accumulation in endothelium leads to vascular dysfunction, thereby providing an in vivo model to delineate the basis of cardio- and cerebrovascular complications associated with Fabry disease (139, 140).

Gaucher disease results from insufficient cleavage of GlcCer and glucosylsphingosine by the lysosomal enzyme, acid β -glucosidase or glucocerebrosidase (GCase) (Fig. 3) (95, 141). A homologous pseudogene (ψ GBA) located 16 kb downstream from the *GBA* gene has complicated the molecular diagnosis of Gaucher disease (142). The phenotypes are a continuum of degrees of involvement but can be divided categorically into neuronopathic (types 2 and 3) and nonneuronopathic (type 1) variants (Table 1). A nonlysosomal β -glucosidase (GBA2) has been identified as participating in GlcCer degradation (143). Inhibition or knockout of GBA2 activity is associated with GlcCer accumulation in testis and impairment in spermatogenesis (144). However, the link of GBA2 to Gaucher disease is unclear. Enzyme replacement therapy has been successfully used to treat Gaucher disease type 1 (145), whereas new substrate synthesis inhibition and enzyme enhance-

ment therapies are in clinical trials to treat type 1 and 3 diseases (146, 147). There are no direct treatments available for Gaucher disease type 2. The Gaucher disease mouse models (Table 1) demonstrate that accumulation of GCase substrates, GlcCer and glucosylsphingosine, begin at embryonic day 13 (148). Also, substrate storage does not affect brain formation but does lead to early and severe degeneration of the brain and disruption of the skin permeability barrier (149, 150). The downstream biological consequences of GlcCer and glucosylsphingosine accumulation are currently being investigated (see section III).

Disorders of ganglioside degradation

Two types of ganglioside degradation disorders are characterized. GM1 gangliosidosis results from deficiency of lysosomal GM1- β -galactosidase (EC 3.2.1.23) that cleaves ganglioside GM1 (Fig. 3, Table 1), its asialo derivative, GA1, galactose-containing oligosaccharides, keratin sulfate, and other β -galactose-containing glycoconjugates including galactosylceramide (151). GM1- β -galactosidase is part of a multi-enzyme complex with α -neuraminidase and "protective protein"/cathepsin A (152, 153) that is assembled posttranslationally during transit through the Golgi (154). There is an inverse correlation between disease severity and residual β -galactosidase activity in GM1 gangliosidosis patients (155). GM1 gangliosidosis mice show severe neurological defects and resemble the human disease (156, 157). Preclinical studies using substrate reduction and gene therapies have been tested in animal models of GM1-gangliosidosis (158–160).

GM2 gangliosidoses are a group of severe neurodegenerative disorders unified by the excessive accumulation of GM2 ganglioside mainly in neuronal cells. GM2 is specifically hydrolyzed by β -hexosaminidase A (EC 3.2.1.52) (Fig. 3). Two genes are required for the formation of this enzyme. The *HEXA* and *HEXB* genes encode the α - and β -subunits, respectively (161, 162). The subunits assemble in the Golgi forming $\alpha\beta_2$ [β -hexosaminidase A (Hex A)] and β_3 [β -hexosaminidase B (Hex B)] (161, 163, 164). The nonallelic *GM2A* gene encodes the GM2 activator protein that complexes with the ganglioside GM2 (165) (see below). Defects in any of these three genes may lead to impaired degradation of GM2 gangliosides and related substrates. Tay-Sachs disease (variant B) is the classic GM2 gangliosidosis and results from the deficiency of the α -subunit that produces the specific loss of Hex A, whereas Hex B is at normal or somewhat increased levels, thereby designated as variant B (161). This specific deficiency in Hex A produces GM2 ganglioside accumulation in brain, because GM2 ganglioside is synthesized in appreciable amounts only in the CNS (Fig. 3). Currently, there is no effective treatment for Tay-Sachs disease, although trials of "molecular chaperone" approaches are underway for the late onset variants with residual Hex A activity (166). Sandhoff disease (variant 0) is caused by mutations of the *HEXB* gene that leads to loss of the β -subunit, resulting in deficiency of both Hex A and Hex B activities (Fig. 3). Unlike Tay-Sachs patients who store mainly GM2-ganglioside, Sandhoff patients accumulate additional N-acetyl

β -galactosyl terminated glycolipids and oligosaccharides in the CNS and viscera (167, 168).

The Tay-Sachs disease mouse model (Hex A^{-/-}) displays a milder phenotype compared with Tay-Sachs patients. This is accounted for by the differences in GM2 catabolism between mouse and human (169). Two pathways have been proposed for GM2 metabolism in mice. One involves GM2 hydrolysis to GM3 mainly by Hex A and GM2 activator, which is the major pathway in humans (Fig. 3) (161). The second one is specific to mice and involves GM2 hydrolysis by sialidase first to GA2 and then to LacCer by Hex B. In Hex A^{-/-} mice with a complete loss of Hex A, GA2 can be degraded by Hex B (169). Unlike Hex A^{-/-} mice that have minor GM2 storage in neurons and no detectable neurological impairment, Sandhoff mice (Hex B^{-/-}) are severely affected. The lack of Hex A and B activities in Sandhoff mice provides an authentic model of acute human Sandhoff disease (169). Substrate reduction therapy with N-butyldeoxygalactonojirimycin (NB-DGJ), an imino sugar that inhibits GCS, has been tested in adult (170) and neonatal (171) Sandhoff mice. NB-DGJ significantly reduced total brain and GM2 ganglioside content when administered from postnatal days 2–5 in Sandhoff mice to a greater extent than in the adult mice. No adverse effects were found with NB-DGJ treatment in either age group. These results indicate that earlier intervention is an effective method for managing GSL LSDs.

Activator protein deficiency

The physiologic importance of sphingolipid activator proteins in GSL degradation has been highlighted in the patients with mutations in the GM2 activator and the prosaposin genes; the latter encodes a precursor of four saposins (A, B, C, and D).

GM2 activator (variant AB) deficiency is caused by mutations that lead to an inability to form GM2/GM2-activator complexes in the presence of normal amounts of Hex A and Hex B (Fig. 3) (165, 172, 173). Mechanistically, the GM2 activator protein is a “liftase” that extracts GM2 from intra-lysosomal membranes making the GM2 terminal N-acetyl-galactosamine accessible to β -hexosaminidase A for cleavage (174). The Gm2a^{-/-} mice store mostly GM2-ganglioside and low amounts of GA2 (175), similar to the Hex A^{-/-} mice, thereby establishing its essential role in the GM2 catabolism (175).

Prosaposin/total saposin deficiency was found in four families and was associated with multiple GSL storage (Table 1) (176–178). Saposin (Sap) A is an *in vivo* activator for GALC (124). Sap B has lipid transfer properties and participates in degradation of sulfatide by ASA, digalactosylceramide, and Gb3 by α -galactosidase A, and GM1 ganglioside and LacCer by β -galactosidases (Fig. 3) (179, 180). Sap C is required for GCase to achieve maximal activity *in vitro* and *ex vivo* and protects GCase from proteolysis (181, 182). In comparison to Sap B, Sap C physically interacts with either phospholipid membrane or acid β -glucosidase to optimize this enzyme’s activity (183, 184). Direct binding of Sap C to the substrate, GlcCer, has not been shown as part of this optimization (185, 186). Individual Sap A,

Sap B, or Sap C deficiencies in humans are rare (181, 187–189). Sap D activates AC for degradation of ceramide. Heteroallelic mutations were identified in the Sap D domain in a patient with an additional mutation in the Sap C domain on a different allele (189). Prosaposin and all four Sap-deficient mice have been developed (98, 190–194). In general, individual Sap deficiencies in mice results in slowly progressive diseases compared with total Sap-deficient mice or their cognate enzyme knockout mice (Fig. 3, Table 1). These could be due to cross-compensation in GSL degradation by multiple saposins (Fig. 1). Proinflammation is a common pathological finding in the CNS from such models because of GSL accumulation. The clinical, pathological, and biochemical phenotypes of prosaposin knockout mice closely resembles those of the human disease, whereas Sap B^{-/-} and Sap C^{-/-} models are more slowly developing than those in humans.

Lipid trafficking disorders

Niemann Pick disease Type C (NPC) is not a disorder directly related to the metabolism of GSLs and gangliosides but rather has major involvement in the egress of free cholesterol from the lysosome (195). NPC is characterized by the accumulation of unesterified cholesterol and other lipids in endosomal/lysosomal compartments that stem from inherited deficiencies of the NPC1 or NPC2 proteins. NPC1 is a transmembrane protein and NPC2 is a soluble protein. Both are involved in intralysosomal cholesterol trafficking (196). Recently, NPC2 was proposed to act as a donor of cholesterol to NPC1 that facilitates cholesterol transport across the lysosomal membrane (196). Loss-of-function mutations in the NPC1 gene also lead to failure of the calcium-mediated fusion of endosomes with lysosomes, resulting in the accumulation of GlcCer, LacCer, and GM2 in late endosomes and lysosomes (195). The exact mechanism of disease causality by either cholesterol (primarily) or GSLs (secondary or primary) accumulation has not been resolved. Elevation of sphingosine was found before cholesterol and GSL accumulation (195). The importance of sphingosine in NPC1 has been addressed recently (197). NPC1-deficient mouse models (NPC1^{-/-}) show similarities with the human clinical NPC1 phenotypes (198). Lipid profile and disease phenotype in NPC1, NPC2, and NPC1/NPC2 double mutant mice were qualitatively and quantitatively very similar, indicating that both proteins operate cooperatively without compensation for each other in trafficking cholesterol to the lysosomes (196, 198–200).

Most GSL LSDs present with neurological phenotypes that begin early in life. The analyses of the mouse models show that the lipid accumulation in the CNS starts very early on in development. Such storage may subtly alter brain and cellular functions. GSL accumulation has been postulated as a trigger to abnormal cellular responses that potentiate the pathological disease process. Although some of the mouse phenotypes differ from the analogous human disease, the biochemical profiles in the GSL synthesis or degradation pathway have provided insight into the pathological mechanisms and GSL flux aberrations. However, systematic analyses of GSL profiles in relation to

early brain development (Fig. 4) and the early molecular events have yet to be elucidated. Such understanding should be useful in defining the initiating and propagating mechanisms that would provide a road map for designing therapeutic strategies.

PATHOLOGICAL CONSEQUENCES OF DISORDERS IN GSL METABOLISM

GSL disorders present complex disease phenotypes and diverse pathological changes. The defects in GSL metabolism affect cellular and molecular functions and lead to abnormalities of the CNS and visceral organs (Fig. 5). The mechanisms leading to this pathology are poorly understood. Are these disorders triggered by accumulated lipid or insults from secondary molecular defects in the cells? Does inflammation potentiate the disease? What causes neuronal drop out? Could lesions result from lack of products as well as from storage of substrate in the GSL pathway? The following section summarizes some of the pathological and molecular findings, which may be preludes to understanding the signature pathways in the GSL diseases.

Evidence of GSL accumulation correlating with disease progression

Disruption of GSL flux is thought to initiate and potentiate LSD phenotypes. The slow accumulation of sulfatide in *Asa*^{-/-} mice does not disrupt myelin formation, which occurs in the first 3 weeks postnatally in the mice but does destabilize myelin integrity in adults (201). Although lysosomal accumulation of Gb3 begins in utero, the signs and symptoms of Fabry disease may not be apparent until childhood or adolescence (202). The association of GlcCer, and, especially glucosylsphingosine, with neuronal

toxicity is evident in humans and mice with Gaucher disease. GSL composition studies show that glucosylsphingosine and GlcCer in Gaucher type 1 brains are substantially lower (3–10%) than that in Gaucher disease type 2 brain (203), suggesting a relationship between substrate accumulation and severity of brain disease. Galactosylsphingosine levels in *twitcher* mice brain progressively increase from 20 to 40 days as does the neurological phenotype (119, 204). Unlike galactosylsphingosine in Krabbe disease, glucosylsphingosine has not been proven in vivo as a direct cause of CNS Gaucher disease. However, a recent glucosylsphingosine storage disease mouse model supports this concept (205). The correlations between disease progression and age-dependent GSL levels also occur in NPC disease (206), Tay-Sachs disease (207), and GM1 gangliosidosis mice (156).

GSL accumulation occurs prior to the onset of a disease phenotype, e.g., glucosylsphingosine accumulation was found at the 11th week of pregnancy in human Gaucher disease (208). Elevated glucosylsphingosine can be detected as early as E13 during neurogenesis in *Gba* knockout mice (208). In *V394L/SapC*^{-/-} mice, increased glucosylsphingosine is present by 14 days, i.e., before the onset of neurological signs at 30 days (Table 1). Abnormal accumulation of galactosylsphingosine in *twitcher* mice is detected in the brain and sciatic nerves at postnatal day 7, before the onset of demyelination, and progressively increases to 100-fold normal levels, predominantly in white matter (209, 210). In normal mice, GM2 is not detectable, but GM2 is elevated at postnatal day 2 in Sandhoff mice before any pathological signs (171). Such early lipid accumulation may trigger molecular alterations, e.g., proinflammation, as shown by global gene expression analyses in Sandhoff disease mice in which upregulation of inflammation-related genes preceded neuronal death (211). Similar temporal analyses of CNS tissues from prosaposin-deficient mice reveal the regionally specific gene expression abnormalities including upregulation of numerous proinflammatory genes prior to neuronal degeneration (212). Although low-level excess of GSLs may not lead to early histopathological changes, they do cause cellular alterations that initiate disease processes leading to eventual end stage disease. A final common pathway in the CNS for the GSL LSDs appears to be varying types and degrees of inflammatory and proinflammatory reactions leading to apoptotic or other types of programmed cell death.

Increased lipid levels at early embryonic stages also correlate with reduced expression of genes for neurotrophic factors [brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF)] and MAPK pathway genes (ERK) in the cortex, brainstem, and cerebellum of neuronopathic Gaucher disease mice at E17.5, E19.5, and P1 (213). The deficiencies in neurotrophic factors (BDNF and NGF) cause sensory neuron degeneration in mice (214, 215). These early abnormalities in lipid and neurotrophic factors correlated with substantial reductions in cellular density, neurodegeneration, and microglial infiltration in *Gba* knockout mouse brains at E13–14 (149, 150, 208). Injection of the GCase inhibitor (cyclopellitol)

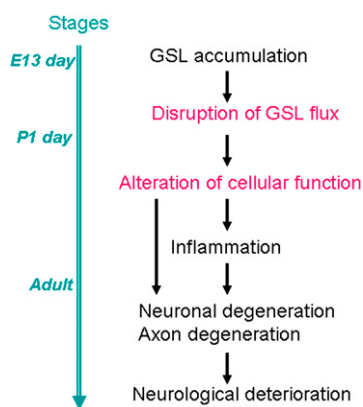


Fig. 5. Hypothetical scheme for neuronal disease progression in the nervous system. Accumulation of GSLs causes abnormalities of neuronal cell functions in mouse models and the human diseases. Abnormal degradation of GSL may disrupt the normal flux of GSL in the cell, which can affect a variety of cellular functions. How the GSL accumulation alters GSL flux and cellular function is largely unknown. The changes of the cellular function may cause neuronal degeneration. Inflammation is often detected before the neuronal and axonal defects and it plays a role in potentiating disease progression. The blue line on the left shows the stage of brain development.

into wild-type mice led to a 17- to 31-fold increase of glucosylsphingosine in brain, liver, and spleen with concomitant neurological abnormalities (216). These findings provide further evidence for the relationship of abnormal substrate levels in GCase deficiencies and neuronopathic phenotypes. These data suggest that a toxic stimulus is present long before birth or at least early in postnatal life, and that therapy begun during infancy may not prevent progressive neurologic damage. In this regard, the conditional experiments of Xu et al. (150) are instructive. A chemically induced model of CNS Gaucher disease was produced by administering a covalent inhibitor, conduritol B epoxide (CBE), of GCase to neonatal mice (150). Wild-type and GCase point mutated mice were given daily injections of CBE from postnatal day 5 to day 11. About 1 day after CBE treatment was stopped, the mice developed neuropathic signs. No mice survived if CBE was continued through day 13. Because the wild-type and mutant GCases have $t_{1/2} \sim 60$ h in cells (217), the neuropathic signs in wild-type mice or those with D409H homozygosity stabilized for the next several months in the absence of CBE. However, with a lower activity mutant, D409V/null, the neuropathic signs progressed to very severe within 2 months; unlike the wild-type and D409H homozygotes, the glucosylceramide levels in brain did not decrease, and there was continuing evidence of neuroinflammation. These data support the concept that specific reconstitution levels of in situ activity must occur at the appropriate developmental time frame to avoid propagation of disease. Furthermore, once established, the neuronopathic disease does not resolve but can be stabilized.

Secondary storage or deficiency of GSLs

Alterations of GSL flux can directly affect membrane composition and disrupt cell homeostasis that may cause secondary storage (38) and potentiate disease processes. Such secondary accumulations of GSLs and gangliosides are common features associated with neuropathology in several LSDs, e.g., Niemann-Pick diseases with GM2 and other GSL accumulation (218–220). Pronounced increases in gangliosides, GlcCer, and LacCer occur in prosaposin deficiency (178, 190) and increases in GM2 and GM3 are evident in the cathepsin D-deficient mouse (221), as they are in the CNS of several mucopolysaccharidoses, MLD, Tay-Sachs disease, and Gaucher disease (222). Importantly, in neurons with secondary gangliosides accumulation, the pathological changes were essentially identical to those in the gangliosidosis (223). Because GM2 and GM3 are precursors of many gangliosides and are the final common metabolites in the ganglioside degradative pathway (224), the discovery of secondary ganglioside accumulation indicates the importance of the endosomal/lysosomal system in the overall regulation of ganglioside expression in neurons.

In the NPC 1 mice, sphingosine accumulation reduces the lysosomal calcium pool and may trigger secondary GSL storage (195). GCase mutations, even in the heterozygous state, are predisposing factors for Parkinson disease (225, 226). It is unknown whether the GSL or other cellular abnormalities determine the association between these two

diseases, but increased α -synuclein deposition and Lewy bodies occur in Gaucher disease type 1 brains (227).

A clear connection has yet to be established between the primary lysosomal defect and the accumulation or deficiency of secondary compounds that become involved in the storage process. Signal transduction derangements and altered trafficking of GSLs in the endosomal/lysosomal system would be possible causes. Regardless of the mechanism, secondary GSL storage compounds appear to be actively involved in disease pathogenesis.

Inflammation

Inflammation, constituting a local response to cellular insult, occurs in visceral and CNS tissues as a general pathological event in GSL and ganglioside LSDs. The excess lipid is causally linked to a proinflammatory response, including activation of the microglial/macrophage system to produce inflammatory cytokines leading to CNS damage. In Gaucher disease type 1, GlcCer accumulates mainly in cells of mononuclear phagocyte origin. Serum levels of macrophage-derived cytokines that are pro and antiinflammatory mediators have been variously elevated (228). They include IL-1 α , IL-1 receptor antagonist, IL-6, TNF α , pulmonary and activation-regulated chemokine, and soluble IL-2 receptor (229–232). Such mediators clearly could play a role in disease progression (233–235). In ASM knockout mice, the majority of genes with elevated expression in lung and brain were those involved in the immune/inflammatory response (236). Activation of microglial cells and astrocytes in CNS and PNS parallels such cytokine increases. Ex vivo, gangliosides activate microglia to produce the proinflammatory mediators nitric oxide and TNF α (237). Alternatively, gangliosides also suppress toll-like receptor-induced proinflammatory cytokine expression (238). In Sandhoff disease mice and humans, macrophage/microglial activation and subsequent cytokine expression result in neuronal dysfunction (211). Similarly, progressive CNS inflammatory changes were coincident with the onset of clinical signs in Tay-Sachs, Sandhoff, and GM1 gangliosidosis mice (239).

Use of antiinflammatory drugs in several GSL LSD models slowed disease progression (240, 241). This indicates that multiple inflammatory pathways participate. Nonsteroidal cyclooxygenase-dependent antiinflammatory drugs (e.g., indomethacin, aspirin, and ibuprofen) extended the lifespans of Sandhoff disease and NPC1 mice (240, 242). In Sap A^{-/-} mice, such treatment suppressed only early, but not later, proinflammation, indicating involvement of the cyclooxygenase pathway, but it is not the major inflammatory pathogenic pathway (Barnes et al., unpublished observations). Strikingly, continuous high levels of estrogen during the pregnancy period of Sap A^{-/-} mice protected against proinflammation and demyelination even though galactosylceramide and galactosylsphingosine continued to accumulate (191, 243). These studies implicate the proinflammatory reactions in the propagation and potentially initiation of GSL-mediated CNS degeneration.

The underlying molecular mechanisms of GSL- or ganglioside-induced inflammation are not fully elucidated. Several disparate results have suggested signaling pathways. Macrophage inducible nitric oxide synthase can be regulated by GSL-mediated intracellular calcium elevation (244). AMP-activated protein kinase plays a role in regulating astrocytic galactosylsphingosine-mediated inflammatory responses (245). Similarly, sulfatide induces inflammatory cytokine production in microglia and astrocytes in addition to stimulating phosphorylation of p38, ERK, and JNK (246). Whole genome transcriptome analyses of prosaposin-deficient mice implicated CEBP δ as a common mediator in the STAT3 proinflammatory pathway (212). GlcCer-derived ceramide may play an antiinflammatory role by mediating p38 δ MAPK activation, p38 δ phosphorylation, and regulation of IL-6 (247); the partial deficiency of GlcCer-derived ceramide in Gaucher disease may release, via p38 δ MAPK, suppression of IL-6 and the consequent downstream inflammatory effects (247). Greater insights into these basic mechanisms could facilitate developing adjunctive approaches to altering the progression of CNS disease in the GSL LSDs.

Several GSLs have roles as immune modulators through invariant natural killer T (iNKT) cell selection that relies on interaction of glycolipid antigens through the MHC class I-related glycoprotein CD1d in late endosome/lysosomes (248, 249). In addition to α -galactosylceramide, the mammalian lipid, isoglobotrihexosylceramide, was proposed as an endogenous glycolipid antigen (249). This has been questioned since the isoglobotrihexosylceramide synthase knockout mice do not have iNKT cell abnormalities (250). Deficiency of iNKT cells occurs in prosaposin deficiency, Sandhoff disease, and NPC1 disease (248, 249), indicating that several GSLs, gangliosides, and lysophospholipids may participate in iNKT cell selection (251), but the role of these cells and the molecular connection remain unclear.

Neuronal degeneration

The majority of GSL LSDs with CNS involvement result from processes that lead to neuronal death (Table 1, Fig. 5). CNS neuronal cell loss and gliosis in Gaucher disease types 2 and 3 correlate with GlcCer and, particularly, glucosylsphingosine levels (252, 253). Neurological deterioration in human GM1 gangliosidosis can span a period from birth up to 30 years; apoptosis is the major mechanism of neuronal death, but this is not universal, because several other cell death mechanisms have been implicated in different LSDs. Apoptosis is well understood and is linked to an unfolded protein response (UPR) that includes upregulation of BiP and CHOP, and activation of JNK2 and caspase-12 (254). GM1 loading of wild-type neurospheres causes depletion of ER calcium stores and activation of the apoptotic pathway, implicating ER GM1 accumulation as an inducer of UPR (254). However, the UPR is not general for GSL LSDs, because UPR is not evident in the Gaucher disease brain (255). Apoptotic cell death does occur in mouse and human Sandhoff and Tay-Sachs diseases that have CNS accumulation of GM2 and GA2 (169, 256). How-

ever, no Hex A or Hex B protein is made in the respective models and, thus, misfolding of a mutant protein cannot be implicated. Apoptotic neuronal death is not evident in several GSL disorders, including prosaposin knockout, Gaucher disease, and individual saposin deficient models. Recently, alterations of autophagy pathways in several LSDs, e.g., NPC1 and Batten disease (257), suggest that impairment of the autophagosome system that can lead to neuron death by as-yet-undetermined mechanisms. Altered calcium homeostasis was described in NPC1 disease (195), ASM knockout mice (102, 109), Sandhoff mice (258) and GM1 gangliosidosis mice (254, 259). A mechanistic link among GSL accumulation, Ca²⁺ homeostasis, and neuronal cell death may be of significance for delineating the neuronal degeneration in GSL disorder diseases.

Purkinje cells are a unique population of neurons that are very vulnerable to pathogenic insults, and their degeneration occurs in several GSL storage disease mice (98, 192, 193, 260, 261). The losses of Purkinje cells in these mice progress chronologically from lobule I to X. In ASM knockout mice, progressive Purkinje cell loss begins at $\sim$ 7 weeks, coincident with the appearance of deficits in motor function (261). Purkinje cell death is detected in 60 day old NPC1 mice that store cholesterol and multiple GSLs (GM2, GM3, GlcCer, and LacCer) (260). Decreased Purkinje cell numbers occur in human Sap C deficiency (262) and Sap C^{-/-} mice (193). Purkinje cell loss is also observed in Sap D-deficient mice (98). LC/MS analyses of cerebella from Sap C^{-/-} or Sap D^{-/-} mice revealed small increases in lactosylsphingosine (LacSph) and LacCer or hydroxy ceramide, respectively (98, 193). By comparison, Sap A^{-/-} and Sap B^{-/-} mice do not exhibit Purkinje cell death, probably due to their selective effects on myelin development (191, 212). Although GM2 storage is detected in Purkinje cells of Sandhoff disease and GM2 activator knockout mice, Purkinje cell death was not reported (169, 175). Thus, some GSLs, e.g., ceramide, GlcCer, and LacCer, or LacSph, may cause Purkinje cell loss, but others may not. Several mouse models unrelated to GSL disorders also present with Purkinje cell loss (263–265), thereby implicating other pathways in degeneration of these cells. The mechanism(s) by which Purkinje cells degenerate are unknown, but negative TUNEL assays in Sap C^{-/-} and Sap D^{-/-} mouse brains suggest that apoptosis is not the major mechanism (243, 266). Recently, an imbalance was proposed between induction and flux through the autophagic pathway in contributing to cell stress and neuronal loss in NPC (267, 268). Neurochemical studies in NPC1 mice demonstrate serotonin and its main metabolite, 5-hydroxy-indoleacetic acid, are altered in the cerebellum and cortex of these mice. The levels of the inhibitory amino acid glycine also were 3-fold higher in the cerebellum of NPC1 and increased L-DOPA was detected in the vermis of the cerebellum. These results implicate changes in neurotransmitters that could be a molecular basis of cerebellar neuropathology leading to Purkinje cell death (269).

Axonal degeneration

Axonal degeneration can precede and lead to neuronal death by the inflammatory, myelination defects, and ab-

normal lipid and protein metabolism (270) that occur in GSL and ganglioside LSDs. In GM1 and GM2 gangliosidosis, axonal spheroids and dendritogenesis correlate with progressive ganglioside accumulation (271). In humans and mice with Sap C deficiency, the soma of neurons and Purkinje cells have normal initial ultrastructure, but inclusion bodies and focal swellings of dendrites develop (193, 262). Dysregulation of autophagosomes in axonal termini has been implicated in axon degeneration (272) but has not been explored in GSL LSDs. The pathological roles of axonal spheroids in several GSL disorders, and possible links to neuronal degeneration, have been discussed in a recent review (273).

Abnormal morphology in the sensory nervous system is a common pathology in the GSL LSDs. Foamy storage material occurs in the dorsal root ganglion of GM1 gangliosidosis, Sandhoff disease, GM2 activator deficiency, Sap B^{-/-}, Sap C^{-/-}, Sap D^{-/-}, prosaposin knockout mice, and NPC1 mice, as well as GM2/GD2 synthase knockouts that lack complex gangliosides (274). The storage of GSLs in dorsal root ganglion causes uncoordinated body movement in those models. GM2/GD2 synthase knockout, *twitcher*, and Sap A^{-/-} mice exhibit degenerative changes in sciatic nerve, including myelin fragmentation and myelin layer separation with resultant synaptic rearrangement (118, 191, 274). GalCer or sulfatide are components of myelin GSL, and their excess accumulation causes demyelination in ASA, Krabbe disease, Sap B-deficient patients, and mouse models (124) (130, 275). In primary neuronal and neuroblastoma cultures, lysosomal accumulation of sulfatide mediates increases in endosomal ceramide that leads to apoptosis (276), thereby implicating GSL flux alterations due to sulfatide storage in neuronal death.

The psychosine hypothesis proposed that lysosphingolipids are responsible for the neuronal disease pathology and cell death mediated through aberrant modulation of PKC pathways (277). The findings that other lyso-lipids, lyso-sulfatide, LacSph, lyso-GM1, lyso-Gb3, and glucosylsphingosine are present in the GSL disorders extended this hypothesis to the lyso-sphingolipid hypothesis (278, 279). Experimental evidence to support this hypothesis is growing. Galactosylsphingosine (psychosine) is undetectable in normal tissues but is at high levels in the brains of Krabbe (globoid cell leukodystrophy) patients and mouse models (*twitcher*) (280). The pathology of infantile Krabbe disease includes severe dys/demyelination caused by apoptotic loss of myelin-forming oligodendrocytes and Schwann cells thought to be linked to accumulation of the highly toxic GALC substrate, galactosylsphingosine (277, 281, 282). Importantly, this was the first disease in which a lysoglycolipid was implicated as a major pathogen and led to the postulation that lysoglycolipids were the toxic agents that were responsible for the disease progression in many GSL storage diseases. In the *twitcher* mouse brain, galactosylsphingosine accumulation leads to alteration in lipid raft structure, which may cause abnormal cellular function in oligodendrocyte and Schwann cells and lead to retrograde axonal degeneration (283). A similar mechanism may be operative in neuronopathic Gaucher disease mice

exhibiting increased CNS glucosylsphingosine levels (205, 208). Axonal degeneration has been correlated with elevated GlcCer and glucosylsphingosine levels (205), and glucosylsphingosine toxicity has been demonstrated in neurons by suppression of neural outgrowth (284). Both GlcCer and glucosylsphingosine modulate Ca²⁺ flux in brain microsomes (285). Defective Ca²⁺ homeostasis, especially increases of Ca²⁺ release, presumably from the ER, has been suggested to be responsible for neuronal cell death (286). However, the direct effect of in vivo GlcCer and glucosylsphingosine levels on neuron and axon degeneration remains to be clarified. Several other lyso-glycolipids have been detected in GSL LSDs and their role and/or mechanism of toxicity provide fertile ground for developing support for this universal lyso-sphingolipid hypothesis.

Electrophysiologic studies in GSL disorders

The application of long-term potentiation (LTP) analyses has provided insight into the pathogenesis and levels of neural involvement of the GSL and lysosomal disorders (193, 212, 287). For such analyses, a constant level of pre-synaptic stimulation is converted to a large postsynaptic output, i.e., LTP, a leading experiment to study learning and memory (288). Sap C^{-/-} and V394L/SapC^{-/-} mice have been tested and exhibit reductions of LTP, together with marked accumulation of GlcCer and glucosylsphingosine, suggesting abnormal synaptic plasticity (193, 205). Sap B^{-/-} hippocampi have sulfatide accumulation and normal LTP, showing that although excess sulfatide induced myelin dysfunction, it does not affect LTP (194). Although the molecular basis for LTP alteration association with GSL levels has not been elucidated, calcium dysregulation has been implicated as having a direct effect on LTP (289), which is mediated through glutamate effects on Na⁺ and Ca²⁺ flux. Indeed, disrupted calcium homeostasis was found in isolated brain microsomes from a Gaucher disease type 2 patient, the Sandhoff disease mouse (285, 290), and the ASM knockout mouse (291). Thus, selected abnormal GSL levels could alter LTP by disruption of calcium-mediated vesicular release of neurotransmitters or related receptor functions, as suggested recently for galactosylsphingosine interference of lipid raft assembly (283). The alteration of LTP in selected GSL disorders also could result from secondary pathology, such as proinflammation and neurotrophin defect (292, 293). Neurotrophins have been suggested to modulate LTP (292). BDNF, a positive effector, deficiency was found in NPC1 mice (294), whereas NGF deficiency occurs in Gaucher disease and Sandhoff disease (213). Such deficits in the brain could lead to impairment of LTP and form a basis of more global cognitive and learning deficits in patients.

THERAPY

Treatments for the GSL LSDs (**Table 3**) focus on two primary approaches to decrease the excess accumulated/accumulating GSLs by increasing their degradation rates or decreasing their synthesis rates. The former centers on increasing the hydrolytic capacity of the GSL enzymes by

TABLE 3. Therapy for disorders of GSL and ganglioside metabolism

Diseases	Therapy	Trade Name	Co.	Pipeline	References
Gaucher disease type 1	ERT	Ceredase	Genzyme	FDA approved (Apr 1991)	(145) Genzyme website
		Cerezyme	Genzyme	FDA approved (May 1994)	
		Velaglucerase	TKT Shire	Phase 3 trials completed FDA, fast track status (Aug 2009)	
	SSIT	prGCD	Protalix	Phase 3 trials completed FDA, fast track status (Aug 2009)	(146, 370)
		Zavesca	Actelion	FDA approved (Jul 2003). EMA approved (Nov 2002). Phase 4 trial results expected in 2010	
		Genz-112368	Genzyme	Entering Phase 3 trials	
Gaucher disease type 3	EET	Plicera	Amicus	EMA approved (Oct 2007). Phase 2 trials completed	(307) (310) ExSAR Web site
		EXR-202 (Ambroxol)	ExSAR	Approved by European and other foreign regulatory agencies for other indications. Planning Phase 1 trials in the US	
				FDA approved visceral components of Type 3 disease (2003)	
	ERT	Cerezyme	Genzyme	FDA approved visceral components of Type 3 disease (2003)	(147)
	SSIT	Zavesca	Actelion	Phase 3 trials underway in UK	www.gaucher.org.uk (295, 343, 371)
	ERT	Fabrazyme	Genzyme	EMA approved (Aug 2001). FDA approved (April 2003). Phase 4 trials	
Late onset Tay-Sach disease	EET	Replagal	TKT Shire	EMA approved (Aug 2001), seeking FDA approval	Amicus Web site (166), clinicaltrials.gov (372) clinicaltrials.gov (372, 373) (171)
		Amigal	Amicus	Phase 3 trials underway	
	SSIT	EXR-101 (Pyrimethamine)	ExSAR	Phase 1 trials temporarily suspended	
		Zavesca	Actelion	Phase 2 trials ongoing	
Sandhoff disease	SSIT	Zavesca	Actelion	Phase 3 trials completed	(158)
		NB-DGJ	Toronto Research Chemicals	Preclinical trials in animal models	
GM1 gangliosidosis	SSIT	NB-DGJ	Toronto Research Chemicals	Preclinical trials in animal models	(158)
NPB	ERT	Recombinant human aSMase (rhASM)	Genzyme	Phase 1 Trials complete April 2009. Phase 3 trials begun for 2010. Preclinical trials in animal models (clearance of lipids in liver, spleen and lung)	Genzyme website (374)
NPC	SSIT	Zavesca	Actelion	EMA approved (2003) Phase 2-3 trials	(305, 375)
Krabbe disease	BMT/stem cell transplantation/ gene therapy			Non-FDA approved for Krabbe disease. Ongoing preclinical trials in animal models	(301, 376, 377)
MLD	BMT/stem cell transplantation/ gene therapy			Non-FDA approved for MLD. Preclinical trials in animal models	(131, 300, 378)
Farber disease	BMT/stem cell transplantation			Non-FDA approved for non-CNS Farber disease	(101)

reconstituting target cells to above-threshold amounts of the specific enzyme function via several different approaches: 1) directly supplying the needed enzyme (so called enzyme replacement therapy or ERT) that can be taken up into target cells and delivered as active enzyme to the lysosome by receptor-mediated endocytosis; 2) indirectly providing enzyme by surrogate factories, transplantation of unaffected cells (e.g., hematopoietic stem cells or induced pluripotent stem cells) or addition of genes to cells that can produce wild-type or greater in vivo levels of the needed enzyme for secretion and internalization by the target deficient cells, so called, metabolic cross correction by cell or gene therapy; and 3) use of small molecules that interact directly with the specific mutant enzyme in

cells to increase its activity in situ by improving trafficking to the lysosome, enhancing its stability, improving its catalytic rate constant, reestablishing proteostasis by diminishing the UPR that a mutant enzyme creates, or a combination of all these mechanisms. This approach has been called molecular chaperone therapy, but is more appropriately termed enzyme enhancement therapy or EET. The current studies and trials with EET use potent competitive inhibitors of selected lysosomal enzymes as the chaperone agents.

Treatments by increasing GSL degradation

The success of ERT in ameliorating many, but not all, of the visceral manifestations of Gaucher disease type 1 has

spurred the development of this approach for other LSDs with major visceral involvement, such as Fabry disease (295). This restriction to visceral disease derives mainly from the inability of intravenously administered enzymes to cross the blood brain barrier in therapeutically significant amounts. Data are now emerging that ligands, other than the typical sugars, i.e., mannose-6-phosphate or mannose, may allow transit of proteins from the circulation across the blood brain barrier, particularly at very high plasma concentrations, as shown in the mouse models of β -glucuronidase or ASA deficiencies (132, 296). Such effects were enhanced by using an amino acid tag to increase the delivery of the enzyme to the brain substance (297). In addition, the use of intraventricular or intrathecal administration of appropriate enzymes could provide for significant therapeutic effects in the CNS. Similar considerations also apply to postneonatal delivery of AAV viral vectors for genetic transduction following intravenous administration. This limitation appears to have been partially overcome by the inclusion of disease-specific peptide ligands into AAV vectors that would then bind to endothelial cells of affected animals and express the needed enzyme, thereby facilitating uptake into affected CNS cells including neurons (298). Such an approach led to widespread distribution of the expressed enzyme in the brain following intravenous administration with resultant biochemical and phenotypic improvement.

Transplantation of hematopoietic, embryonic, neural progenitor, or induced pluripotent stem cells with or without transduced additional genes is being used to deliver the needed therapeutic enzymes (299–303). Such cells can either add to existing cell populations in the brain, i.e., glia or neurons, and/or act as metabolic factories for the enzymes that can then be secreted and taken up by enzyme deficient cells in the CNS and other organs to varying degrees. Such approaches have had varying success in altering the CNS phenotypes because of the timing and nature of the underlying CNS pathology in the GSL LSDs. The degree of reversibility is determined by the time of treatment and/or the overall distribution of the therapeutic cells and/or enzyme to different regions of the brain (see below).

Treatments by decreasing GSL synthesis

The approach of inhibiting the synthesis of GSLs to treat the corresponding diseases was first suggested by Norman Radin some 30 years ago. The general concept is to inhibit GCS, because GlcCer is the precursor of the lipids in the majority of GSL LSDs, but more specific enzymes for the synthesis of higher GSLs and gangliosides could also be targeted. Because the complete deficiency of GCS is lethal in utero, partial inhibition of GlcCer synthesis is the goal with the intent to reestablish a balance between synthesis and degradation. For this approach to work, either residual, albeit low, enzyme activity or an alternative pathway for GSL disposal is needed. This approach has been termed Substrate Reduction Therapy. However, this is a misnomer, because the goal of all the therapeutic approaches is to reduce substrate accumulation; a better

term would be substrate synthesis inhibition therapy or SSIT.

Oral SSIT with N-butyl-deoxynojirimycin (NB-DNJ) (miglustat or Zavesca; Actelion, Basel Switzerland) has been approved by the EMEA and FDA for treatment of Gaucher disease type 1 patients who cannot tolerate ERT (147, 304). This drug is in Phase 3 trials for Sandhoff disease (158) and for NPC1; miglustat has been approved by EMEA (305). The therapeutic effects of NB-DNJ are less than achieved with intravenous ERT using mannose-terminated GCase (304). Use of NB-DNJ in Gaucher disease type 3 patients with established CNS disease failed to show significant improvement in neurological signs, specifically abnormal eye movements (147). However, sufficient neurological effects were found in NPC1 disease for EMEA approval for that condition (306) (<http://nnpdf.org>; http://www.emea.europa.eu/pdfs/human/opinion/Zavesca_33596508en.pdf). More recently, a more potent GCS inhibitor (Genz-112638) based on the parent compound, 1-phenyl-2-decanoylamino-3-morpholino-1-propanol, has shown major clinical and biochemical effects in visceral organs from humans and mice with Gaucher disease type 1 variants (307). Importantly, the lungs were substantially cleared of GlcCer storage in the mice, an organ that has poor biochemical response to ERT (307). Genz-112638 is a Pgp substrate and, although it rapidly crossed the blood brain barrier, significant levels of the drug are not achieved due to rapid removal by Pgp (307). Phase II data of Genz-112638 from the human trial show similar safety and initial efficacy at 6–12 months to ERT with imiglucerase (308).

A clear advantage of drugs used for SSIT and EET is their ability to cross the blood brain barrier in potentially therapeutically significant amounts. Furthermore, the EET approach to re-engineer the mutant enzyme in situ for better function has additional advantages of oral administration and avoiding immunological reactions and the necessity of specific ligands for receptor mediated endocytosis. The first generation of these agents for the GSL storage diseases are primarily iminosugars that are either 5- or 1- deoxy or 3,4-dideoxy glycoside derivatives of glucose or galactose, and they are potent reversible competitive inhibitors of specific lysosomal hydrolases. The current agents, isofagomine [3,4-dideoxy-5- (hydroxymethyl) piperidine] and DGJ (1-deoxy galactonojirimycin), have shown significant ability to enhance the activity of a great variety of different mutant GCases or α -galactosidases A, respectively, in tissue culture and in peripheral blood leukocytes of affected patients with Gaucher or Fabry disease, respectively (309–311). Clinical trials in Fabry disease are ongoing with DGJ, but the development of isofagomine has been stopped due to the lack of clinical effect in all but one patient with Gaucher disease type 1 (http://files.shareholder.com/downloads/AMTX/763312895_x_0_x_322616/e986f511-4f40-4412-972d-068e6d7dd058/FOLD_News_2009_10_2_General.pdf). Clearly, alternative drugs with different targets on the mutant enzymes might have greater clinical effects.

For CNS LSDs, early therapeutic interventions have been shown to improve diseases outcome in several LSD mouse models. NPC1 mice treated with cyclodextrin at P7 versus P21 showed longer lifespans (312). NB-DGJ treatment started at P2, when GM2 accumulation was detected, significantly reduced total gangliosides in Sandhoff disease mice (171). Isofagomine treatment on V394/SapC^{-/-} mice started in utero (E15) extended lifespan versus treatment started at P21 (Sun et al., unpublished observations). The dosage used in the early intervention at embryonic stage is critical, because they may be toxic in utero effects. Understanding the GSL profile and function during brain development (Figs. 4 and 5) will be important to design the therapy and to define the timing for effective treatment of CNS LSDs without disrupting normal brain development.

The disease stage and pathogenic mechanisms of the CNS GSL storage diseases are of major importance to the development of therapies. Many of the studies of ERT, cell transplantation, and gene therapies have shown promising results in animal models in which the disease processes may be reversible, e.g., GSL storage is present in selected CNS cells, but the neurons remain viable. In such cases, one can envision the reestablishment of GSL flux in such cells might lead to recovery of their function and a positive effect on phenotype. In comparison, if the disease process has neuronal loss as a major pathogenic mechanism, recovery or even stabilization might be impossible; e.g., in neuronopathic Gaucher disease mice, neither complete enzyme reconstitution within neurons nor provision of microglia with wild-type GCase activity led to restoration or rescue of the CNS disease (150). Neuronal death is predominant in these models. Much more detail of the pathogenic mechanisms will be required for development of disease-specific appropriate therapeutic approaches.

CONCLUSIONS AND PERSPECTIVES

Advances in understanding the pathophysiology and treatment of the GSL diseases hinge upon molecular insights into the basic and initial mechanisms of disease causation. Elucidation of the earliest molecular events caused by the subclinical accumulation of GSLs could provide initial targets that are fundamental to interfering with the propagation of the neuronal death pathways. Such insights are critically important as the primary neuronal disease, cell death versus GSL storage, would require highly different intervention strategies and timing of initiation. Recent proposals that retrograde axonal degeneration due to lipid raft aberrations provide additional pathways and targets for therapy and for the understanding of the role of GSLs in cell development and physiology. These rare diseases continue to provide platforms for the general understanding of GSL biochemistry and therapy for many diverse and more common diseases. 

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