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Review

Sphingolipid metabolism diseases

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Abstract

Human diseases caused by alterations in the metabolism of sphingolipids or glycosphingolipids are mainly disorders of the degradation of these compounds. The sphingolipidoses are a group of monogenic inherited diseases caused by defects in the system of lysosomal sphingolipid degradation, with subsequent accumulation of non-degradable storage material in one or more organs. Most sphingolipidoses are associated with high mortality. Both, the ratio of substrate influx into the lysosomes and the reduced degradative capacity can be addressed by therapeutic approaches. In addition to symptomatic treatments, the current strategies for restoration of the reduced substrate degradation within the lysosome are enzyme replacement therapy (ERT), cell-mediated therapy (CMT) including bone marrow transplantation (BMT) and cell-mediated "cross correction", gene therapy, and enzyme-enhancement therapy with chemical chaperones. The reduction of substrate influx into the lysosomes can be achieved by substrate reduction therapy. Patients suffering from the attenuated form (type 1) of Gaucher disease and from Fabry disease have been successfully treated with ERT.

Keywords: Ceramide; Lysosomal storage disease; Saposin; Sphingolipidose

Contents

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1. Sphingolipid structure, function and biosynthesis

Together with glycerophospholipids and cholesterol, sphingolipids are building blocks of eukaryotic membranes. They are characterized by the presence of a sphingoid base within the hydrophobic part of the molecule. In sphingomyelin and the glycosphingolipids, a phosphorylcholine or a carbohydrate moiety are bound to the terminal hydroxyl group of ceramide (*N*-acylsphingosine), respectively (Fig. 1) [1].

Glycosphingolipids display a high structural diversity and can be classified into series, which are characteristic for a group of evolutionary related organisms [1]. On cellular surfaces, glycosphingolipids form characteristic patterns that are species- and cell-type specific and change with cell growth, differentiation, viral transformation, ontogenesis, and oncogenesis [2]. In the plasma membrane, they are believed to segregate into pre-existing microdomains [3,4], which are enriched in glycosyl-phosphatidylinositol-anchored proteins, sphingomyelin, and cholesterol. These so-called lipid rafts [5,6] might constitute the physiological surroundings of many membrane proteins.

Glycosphingolipids are essential for development and survival of multicellular organisms [7]; they play a role in cell adhesion phenomena [8,9] and in the regulation of membrane proteins. Thus, absence of ganglioside GM3 in vivo leads to enhanced insulin receptor phosphorylation and increased insulin sensitivity [10]. Correct sphingolipid processing is also vital for the barrier function of the human skin [11], where ceramides of the *stratum corneum* contribute to the water permeability barrier. Skin ceramides occur in free form [12], or covalently linked to proteins of the cornified envelope [13]. In addition to disorders caused by alterations of sphingolipid metabolism, sphingolipids are involved in a variety of diseases [14]. In infectious diseases, sphingolipids serve, e.g., as pathogen receptors [15–17] and can control pathogen infection and host defense [18]. In the immune system, glycosphingolipids play a role as antigens (ABO-System, Forssman), but can also stimulate the generation of autoantibodies in postinfectious autoimmune diseases like Guillain–Barré or Miller–Fisher Syndromes [19,20]. They are ligands for CD1d-restricted natural killer T-cells (NKT-cells) [32]; for example, the highly immunogenic, non-human α-galactosylphytoceramide activates a subset of NKT cells. This glycosphingolipid from the marine sponge *Agelas mauritianus* [21] has gained much interest as a potential drug in the treatment of cancer and autoimmune diseases [22,23]. An endogenous sphingolipid ligand for CD1d and important for the maturation of NKT-cells appears to be isoglobotriaosylceramide. This was demonstrated by a deficiency of invariant NKT-cells in β-hexosaminidase B-deficient mice, the animal model of Sandhoff diseases, which cannot convert isoglobotetraosylceramide into isoglobotriaosylceramide [24].

1.1. Less precisely defined is the role of sphingolipids in cancer [25]

Intermediates of sphingolipid metabolism appear to be involved in the transduction of extracellular signals into the interior of cells. Ceramide can be released from sphingomyelin or by *de-novo* synthesis in response to substances like vitamin D3, tumor necrosis factor α, γ-interferon, or interleukin 1. In most cell types, ceramide mediates antimitogenic responses such as cell differentiation, cell cycle arrest, cell senescence or apoptosis [26]. Also other intermediates of sphingolipid metabolism such as sphingosine, sphingosylphosphorylcholine [27], sphingosine-1-phosphate and ceramide-1-phosphate [28,29], are regarded as signalling substances. Accordingly, the benefit of pharmacological interference with sphingolipid-mediated processes has been investigated for several diseases [30,31].

Not only sphingolipids–including the signalling substances sphingosine-1-phosphate and ceramide–contribute to many physiological and pathological processes, but also the proteins operating in the sphingolipid pathway can serve different functions. For example, sphingolipid activator proteins are required for the presentation of lipid antigens to CD1 molecules [32].

1.2. Biosynthesis

The first steps of glycosphingolipid biosynthesis lead to the formation of ceramide at the cytoplasmic face of the ER

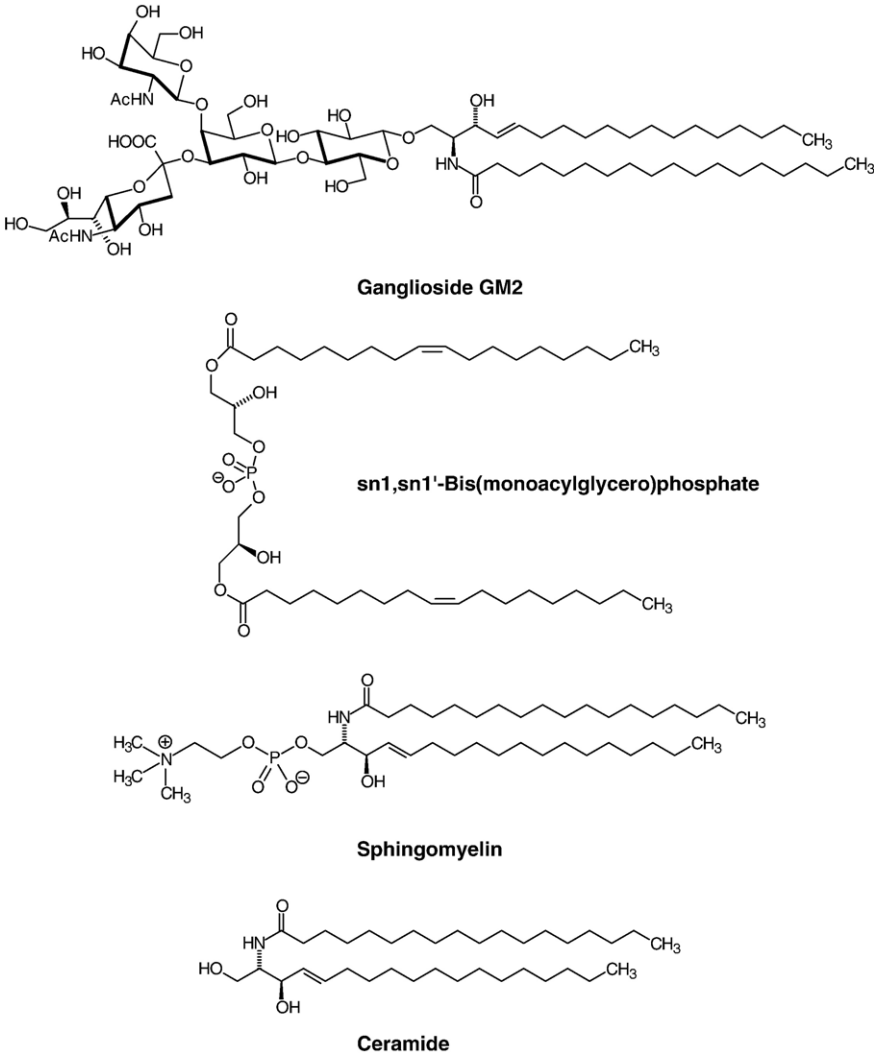

Fig. 1. Structures of ganglioside GM2, bis(monoacylglycero)phosphate (BMP, LBPA), sphingomyelin, and ceramide.

membrane [1,33]. From there, ceramide is transferred to the Golgi apparatus with the aid of a transfer protein, CERT [34]. On the cytosolic leaflet of the Golgi membrane, glucosylceramide is formed and translocated to the luminal face, presumably by multidrug transporters [35,36]. Subsequent glycosidation reactions are catalyzed by glycosyltransferases in the Golgi apparatus [37,38]. In the case of gangliosides, the limited specificity of some of the transferases [39] gives rise to complex patterns on the cell surface within a combinatorial biosynthetic pathway [40]. Genetically modified mice deficient in glucosylceramide synthase die as early as embryonic day 7.5 [41], indicating a role of glucosylceramide or higher glycosphingolipids in embryogenesis. In most cell types, de novo-biosynthesis constitutes only a minor pathway for sphingolipid formation; metabolic labelling studies indicate that the majority of glycosphingolipids are formed by the recycling of building blocks within a salvage-pathway [42]. After their biosynthesis, glycosphingolipids reach the plasma membrane through vesicular exocytotic membrane flow.

By far, most of the human diseases associated with sphingolipid metabolism are degradation disorders. There are only a few human diseases known so far that are caused by alteration of biosynthetic enzymes. Hereditary sensory neuropathy, type I, is caused by mutations in the SPTLC1 gene leading to an increase in serine palmitoyltransferase activity, subsequent elevation of ceramide levels, and enhanced neuronal apoptosis [43,44]. A deficiency of a biosynthetic enzyme associated with a human disease is reported for lactosylceramide α2,3 sialyltransferase (GM3-synthase). Deficiency of this enzyme causes an autosomal recessive infantile-onset symptomatic epilepsy syndrome [45]. There is also some evidence that type 9 of the neuronal ceroid lipofuscinosis might be caused by a defect of a sphinganine acyltransferase regulator protein and subsequent impairment of dihydroceramide biosynthesis [46].

2. Glycosphingolipid catabolism

The lysosomal degradation of membranes requires transport and lipid sorting steps, before glycosphingolipids and other membrane components can be degraded [47]. The cleavage products, monosaccharides, sialic acid, fatty acids, and

sphingosine, are able to leave the lysosome. This is mainly facilitated by transporters localized in the endosomal and lysosomal perimeter membranes.

2.1. Topology

Lysosomes digest extracellular and intracellular macromolecules that enter the organelle by endocytosis, phagocytosis, or autophagy. Plasma membrane components reach the lysosomal compartment via endocytotic vesicular flow [48], either as intraendosomal and intralysosomal membranes, or as part of the limiting membrane [49] (Fig. 2). Both membrane pools differ in their lipid and protein composition. The major part of membrane digestion proceeds on the surface of internal membrane structures. In contrast to this pool, the perimeter membrane is protected from degradation by a glycocalix (Fig. 2) composed of glycoproteins amply glycosylated with lactosamine units [50]. Intraendosomal and intralysosomal membranes of appropriate lipid composition can be degraded by lysosomal hydrolases in the presence of sphingolipid activator proteins and other lipid transfer proteins.

These internal membranes have been initially observed in cells from patients with sphingolipid storage diseases such as GM1 gangliosidosis [51] or combined sphingolipid activator protein deficiency [52], where they accumulate as multivesicular storage bodies. Sphingolipid activator proteins are required for membrane digestion, therefore, the essentials of lysosomal glycolipid digestion [47] are briefly discussed here.

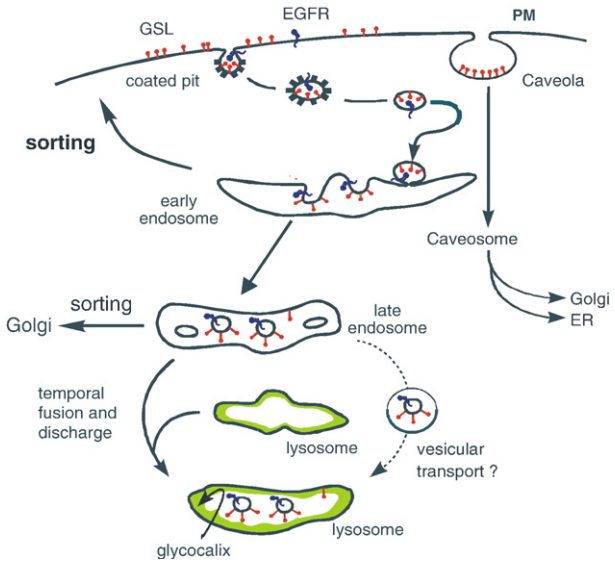

Fig. 2. Endocytosis and lysosomal digestion of membranes. Glycosphingolipids (GSL) are highlighted on the plasma membrane (PM) and on internal membranes [47]. Parts of the plasma membrane including GSLs are incorporated into the membrane of intraendosomal vesicles and membrane structures during endocytosis. The vesicles reach the lysosomal compartment when late endosomes are transiently fused with primary lysosomes. They are the sites of membrane digestion. The lysosomal perimeter membrane is protected from degradation by a thick glycocalix. EGFR: epidermal growth factor receptor.

2.2. Sphingolipid activator proteins

Monosaccharide residues from the nonreducing end of the oligosaccharide part of the glycosphingolipids are sequentially cleaved off by the action of acid exohydrolases. The substrates of these enzymes are embedded in intraendosomal and intralysosomal membranes [53–55], whereas the enzymes are dissolved in the lysosol. In vivo, GSLs with less than four sugar residues [56] are only degraded in the presence of sphingolipid activator proteins (SAPs). In vitro, these SAPs can often be replaced by detergents. SAPs mediate the interaction between the membrane bound lipid substrate and the water-soluble enzyme or activate the enzyme directly. In vivo, enzymatic hydrolysis of most membrane-bound sphingolipids is also stimulated by anionic lysosomal lipids, especially by bis-(monoacylglycero)-phosphate (BMP, lysobisphosphatidic acid), which concentrates in the inner membranes of lysosomes [57]. As indicated in Fig. 3, many catabolic reactions in this pathway require the presence of an activator protein.

To date, five sphingolipid activator proteins (SAPs) are known: the GM2 activator protein and the four saposins (Saps) -A, -B, -C, and -D [79]. The GM2-activator is an essential cofactor in the *in-vivo* degradation of ganglioside GM2 by β-hexosaminidase A [58]; the inherited deficiency of this protein leads to the AB variant of GM2 gangliosidoses (Fig. 4).

For the presentation of ganglioside GM2 or related glycosphingolipids, e.g., GM1 [56], to the active site of the degrading enzyme, the GM2-activator has to insert into the bilayer of intralysosomal lipid vesicles. Results from in vitro experiments indicate that the GM2-activator can insert into a model membrane only below a critical lateral pressure of 15 to 25 mN/m [59]. The lipid recognition site of the activator interacts with the substrate, so that the hydrophobic ceramide portion of the substrate becomes embedded in the hydrophobic cavity of the activator protein. After a conformational change of the lipid-loaded activator increasing the water-solubility of the complex, this is released from the membrane, and ganglioside GM2 is presented to the enzyme and is subsequentially degraded [60].

The Saps or saposins A–D are glycoproteins with molecular weights of 8–11 kDa [79]. They belong to a family of saposin-like proteins with lipid binding- and membrane perturbing properties [32]. Although the four Saps share a high degree of homology [61] and some properties, they act differentially and show different specificity. This leads to the different phenotypes of the different Sap deficiencies (see Section 3.12) Besides their function as enzyme cofactors, sphingolipid activator proteins play an important role in lipid antigen presentation. They participate in the loading of lipid antigens to immunoreceptors such as human CD1b [62], human [63], and mouse CD1d [64].

2.3. Intralysosomal membranes

The degradation of intralysosomal membranes depends on their lipid composition. Intralysosomal membranes, but not the lysosomal perimeter membrane, are enriched in bis-(monoacylglycero)-phosphate (BMP), which is biosynthetically

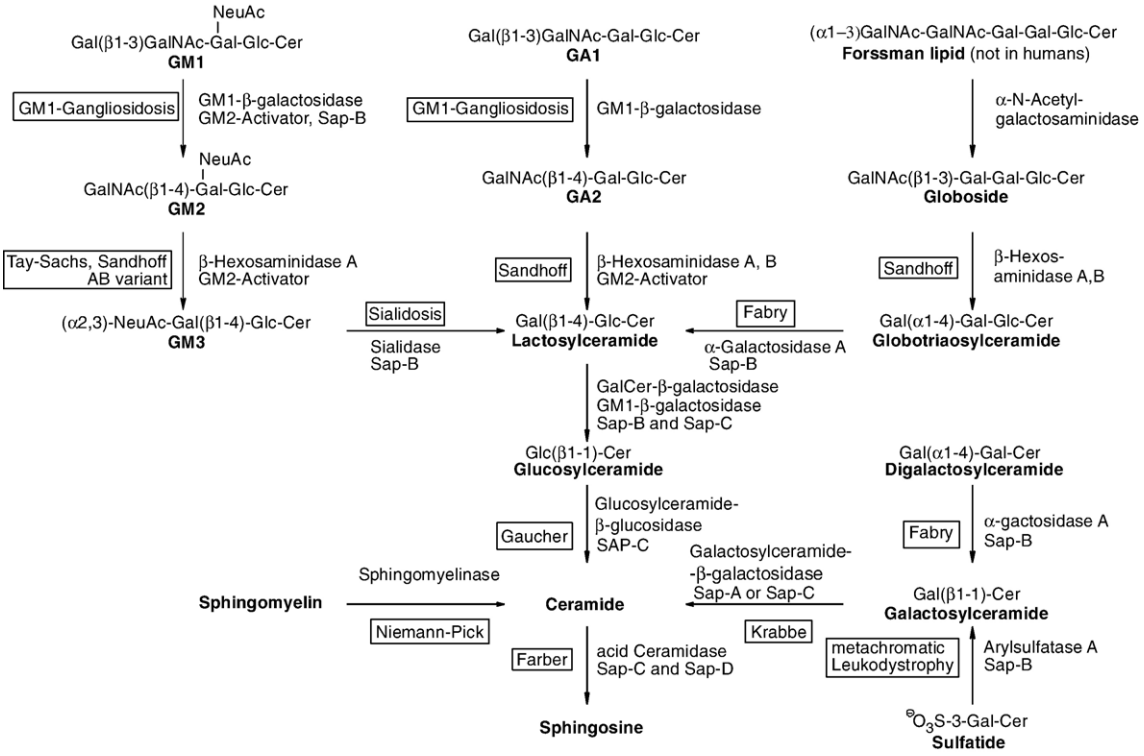

Fig. 3. Degradation of selected sphingolipids in the lysosomes of the cells [47]. The eponyms of individual inherited diseases are given. Activator proteins required for the respective degradation step in vivo are indicated. Variant AB, AB variant of GM2 gangliosidosis (deficiency of GM2-activator protein); Sap, saposin.

formed during the degradation of phosphatidylglycerol and cardiolipin, [65,66] presumably on the surface of these vesicles. Due to its unusual *sn1*,*sn*1′-configuration, BMP has a sufficiently long lifetime in spite of the presence of the lysosomal phospholipases [67]. Other anionic lipids like phosphatidylinositol [68] and dolichol phosphate [69], albeit in smaller amounts than BMP, are also found within the lysosomal compartment. Furthermore, cholesterol is almost absent in lysosomes [57]. High amounts of BMP and low amounts of cholesterol in internal lysosomal membranes appear to be required for the degradation of glycosphingolipids. Decreasing amounts of cholesterol in artificial vesicles increase the membrane-perturbing capability of Sap-A (Locatelli Hoops, S., Klingenstein, R. and Sandhoff, K., unpublished), Sap-B, and Sap-D (Remmel, N. and Sandhoff, K., unpublished). The presence of BMP in these vesicles increases the ability of the GM2-activator [70], Sap-B, and Sap-D (Remmel, N. and Sandhoff, K., unpublished) to solubilize lipids. BMP also stimulates degradation of sphingomyelin by acid sphingomyelinase [186], ceramide by acid ceramidase [198], and glucosylceramide by glucosylceramide-β-glucosidase [137]. In addition, negatively charged lysosomal lipids drastically stimulate the

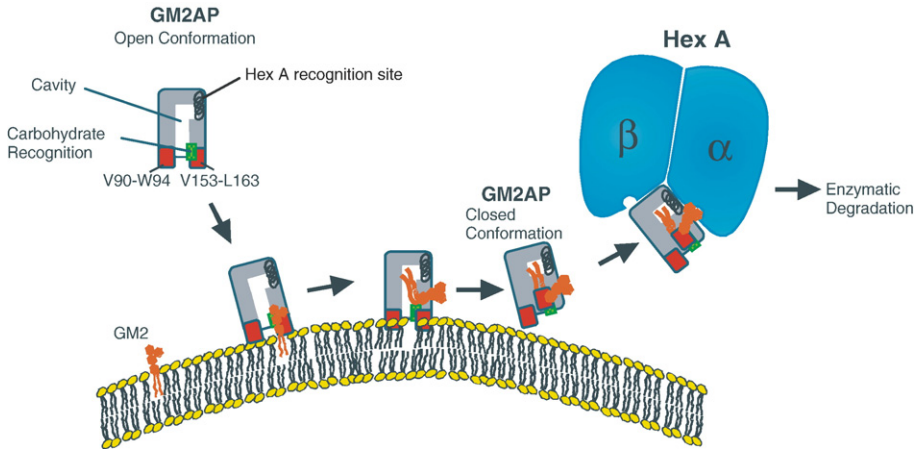

Fig. 4. Model of GM2-activator-stimulated hydrolysis of ganglioside GM2 by human β-hexosaminidase A [60]. The glycolipid binding site is lined by two hydrophobic (V90-W94 and V153-L163) surface loops and a single short helix. The most flexible of the loops (V153-L163) controls the entrance to the hydrophobic cavity allowing both, an open and closed conformation. GM2AP=GM2-activator protein, Hex A=β-hexosaminidase A.

interfacial hydrolysis of membrane-bound ganglioside GM1 by GM1-β-galactosidase [137]. A similar stimulation was demonstrated for the degradation of ganglioside GM2 by β-hexosaminidase A [70], and for the sulfated gangliotriaosylceramide SM2 by β-hexosaminidases A and S [103] in the presence of the GM2-activator protein. Furthermore, in the presence of Sap-C, a drastic enhancement of glucosylceramide degradation by glucosylceramide-β-glucosidase can be produced by negatively charged model lipids such as phosphatidylserine, phosphatidylglycerol, and phosphatidic acid [71,72,133].

3. Sphingolipidoses

Together with mucopolysaccharidoses, mucolipidoses, glycoprotein, and glycogen storage diseases, sphingolipidoses belong to the lysosomal storage diseases (LSDs) [73–77]. With a collective frequency of 1 in 7000–8000 live births [78], the LSDs are rare disorders. About 40 genetically different forms are known. Defects in enzymes, cofactors (such as sphingolipid activator proteins [79]), but also in the targeting or transport systems involved in the degradation process can lead to a LSD.

The sphingolipidoses [80] are a group of inherited diseases, which are caused by defects in genes encoding proteins involved in the lysosomal degradation of sphingolipids. The diseases are usually named according to the identity of the storage material [75,81]. The mode of inheritance is autosomal recessive with the exception of Fabry disease [115]. According to the strictly sequential degradation pathway of glycosphingolipids in humans, defects for almost every step in the degradation of these lipids have been described. An exception is the degradation of lactosylceramide, which can be degraded by two different enzyme/activator systems [82]. Therefore, no single enzyme defect is known that leads to isolated lactosylceramide storage. Together with other sphingolipids, however, lactosylceramide accumulates when various cofactors are absent, as it is the case in prosaposin deficiency [209].

Most enzymes and cofactors deficient in the sphingolipidoses have been characterized, their genes have been cloned, and animal models of most of the sphingolipidoses have been created by targeted disruption of the respective genes in mice [83,84]. Although many of the different sphingolipidoses have been sub-classified into types that differ in course and onset, there is a clinical continuum for these diseases. As it will be discussed below, this is largely caused by differences in the residual activities of the defective proteins.

3.1. GM1-gangliosidosis

GM1-gangliosidosis is caused by an inherited deficiency of the lysosomal enzyme GM1-β-galactosidase [85,86]. GM1-β-galactosidase is a protein of 64 kDa, which is derived from an 88-kDa precursor (review: [86]). It occurs as part of a lysosomal multienzyme complex, together with sialidase, the so-called protective protein [87], cathepsin A, and *N*-acetylaminogalacto-6-sulfate sulfatase [88]. GM1-β-galactosidase catalyses the hydrolysis of ganglioside GM1 to GM2 in the presence of either the GM2-activator protein or Sap-B [56]. According to the substrate specificity of the variant enzyme, an inherited defect can also lead to another disorder, Morquio disease, type B. Storage of galactosylceramide-β-galactosidase substrates, galactosylceramide and lactosylceramide, does not occur. Three clinical forms of GM1-gangliosidosis can be distinguished: In infantile (type 1) GM1-gangliosidosis, developmental arrest and progressive deterioration of the nervous system occur in early infancy. Characteristic symptoms are exaggerated startle responses to sound, a macular cherry-red spot, hepatosplenomegaly, rigospasticity associated with seizures, and generalized skeletal dysplasia. Most patients die within the first 2 years of life.

The late infantile/juvenile form (type 2) is characterized by progressive neurologic symptoms in children, and the adult/chronic form (type 3) occurs in young adults. In adults, extrapyramidal signs frequently presenting as dystonia are the most common neurologic manifestations. Dysmorphic changes are less prominent or absent in these clinical forms.

Apart from the ganglioside GM1, other enzyme substrates also accumulate, such as glycolipid GA1 [89], oligosaccharides from glycoproteins, and intermediates of keratan sulfate degradation. These substances are stored in different organs, according to their major site of biosynthesis. Massive lysosomal GM1-storage in neurons leads to degeneration of the nervous system. On the other hand, formation of meganeurites and ectopic dendrogenesis are presumably due to the antineurotoxic [90], neuroprotective, and neurorestorative properties of ganglioside GM1 in the plasma membrane. Like in other storage diseases, a neuroinflammatory response has been implicated in the pathogenesis of GM1-gangliosidosis [249]. Like in other neurodegenerative diseases [258], an unfolded protein response has been identified in the mouse model of the disease [91]: Mislocalized GM1 activates ER-chaperones and leads to neuronal apoptosis. The severity and progression of the disease correlates with the residual enzymatic activity in cells and body fluids [92].

Gene mutations identified in GM1 gangliosidosis and Morquio B disease include missense/nonsense mutations, duplications/insertions, and insertions causing splicing defects. Neither the type, nor the location of mutations in the gene of GM1-β-galactosidase could be correlated to the phenotype of the patients. Also, mutations within the protective protein can cause accumulation of ganglioside GM1 via destabilization of the β-galactosidase. An engineered mouse model resembling the neurological phenotype of human GM1-gangliosidosis is available [93]. Spontaneous animal models of this disease, such as feline and canine, are also known [86]. A successful treatment of this disease is not available to date; bone marrow transplantation did not correct the neurological phenotype of the juvenile variant of the disease [94]. A chemical chaperone therapy for the brain pathology has been suggested [95], and substrate reduction therapy reduces storage levels in the brain of the mouse model of the disease [96].

Morquio type B disease clinically resembles a mild phenotype of Morquio A disease, where keratan sulfate accumulates due to *N*-acetylgalactosamine-6-sulfatase

deficiency. Like GM1-gangliosidosis, Morquio type B is due to the inherited defect of GM1-β-galactosidase. It is characterized by the predominant storage of keratan sulfate and oligosaccharides with terminal galactose residues. Patients show generalized skeletal dysplasia. Involvement of the nervous system and hepatosplenomegaly are absent. The differences between GM1 gangliosidosis and Morquio B disease can be attributed to a lower affinity and activity of β-galactosidase variants towards substrates with Gal-β1,4-GlcNAc motifs in Morquio patients compared to the Gal-β1,3-GalNAc motive present in ganglioside GM1 [97].

3.2. GM2-gangliosidoses

The GM2-Gangliosidoses [98,99] are caused by defects in degradation of ganglioside GM2 and related glycolipids leading to accumulation of these lipids, most fatally in neuronal cells. GM2 is degraded by cleavage of the β-glycosidic linkage between the *N*-acetylgalactosaminyl residue and the galactose residue by β-hexosaminidases. This reaction requires the GM2 activator protein in vivo. The three lysosomal β-hexosaminidases differ in the combination of their two subunits (α and β), and in their substrate specificity. β-Hexosaminidase A (αβ) cleaves off terminal β-glycosidically linked *N*-acetylglucosamine- and *N*-acetylgalactosamine residues from negatively charged and uncharged glycoconjugates by a retaining double-displacement mechanism. The enzyme has two active sites, one on the α-chain, and the other on the β-chain [100]. β-Hexosaminidase B (ββ) predominantly cleaves uncharged substrates like glycolipid GA2 and oligosaccharides with terminal *N*-acetylhexosamine residues. The crystal structure of human β-hexosaminidase B [101,102] has been solved and shows the two active sites at the homodimer interface, where most of the alterations due to mutations leading to Sandhoff disease are located. β-Hexosaminidase S (αα)) is of secondary significance for GM2 degradation, but it contributes to the degradation of glycosaminoglycans and sulfated glycolipids [103].

The inborn deficiency of the GM2-activator as well as the deficiency of the α- or β-chain of the β-hexosaminidase isoenzymes leads to one of the three different variants of this disease that are named according to the isoenzyme remaining intact. The B-variant is due to an α-chain deficiency, and the subsequent deficiency of hexosaminidases A and S, but with normal hexosaminidase B. Its infantile form is usually called Tay–Sachs disease [104]. The 0-variant, or Sandhoff disease, is caused by the deficiency of the β-chain and the resulting deficient activity of β-hexosaminidases A and B, but remaining activity of β-hexosaminidase S. The AB-variant is a consequence of mutations in the GM2-activator gene and is characterized by normal activities of β-hexosaminidase A, -B, and -S towards the natural substrate GM2 in detergent-containing enzyme assays.

3.3. Tay–Sachs disease

Clinically, the B-variant of GM2-gangliosidoses can be subclassified into infantile, juvenile, chronic, and adult onset forms.

The infantile form, known as Tay–Sachs disease, has a higher prevalence among Ashkenazi Jews with a heterozygote frequency of 1:27. Affected children are normal at birth and show first symptoms, such as mild motor weakness and increased startle reaction between 3 and 6 months of life. Weakness, hypotonia, poor head control, and decreasing attentiveness are observed and visual symptoms appear. A finding in most cases is a cherry red spot in the central retina of the patients. Motor, mental and visual abilities decline rapidly after about 10 month of age. Macrocephaly and seizures are common in the second year. Further deteriorations lead to a vegetative state. Death often occurs between the second and fourth year of life.

In the juvenile form, the first motor symptoms are noted between 2 and 6 years of age, and death occurs at the age of 10 to 15 years. At the end of the first decade, loss of speech, increasing spasticity, seizures, loss of vision, and progressive dementia are common manifestations. A vegetative state is finally reached and the patients die frequently as a consequence of intercurrent infections.

In the chronic form, clinical onset is between 2 and 5 years of age. Symptoms include abnormalities in gait and posture. With advanced age, distinct neurological symptoms appear. Mental and verbal intelligence as well as sensory modalities remain intact. The patients can reach an age of 40 years.

In the adult form, the symptoms are very heterogeneous. Neurological disorders such as spinal muscular atrophy and psychoses may be mimicked. Nevertheless, intelligence and visual capability are not affected.

The B1-variant of GM2-gangliosidoses [105,106] differs enzymatically from the B-variant by an altered substrate specificity of the mutated β-hexosaminidase A. While no activity is detected towards the natural substrate ganglioside GM2 and negatively charged synthetic substrates, synthetic uncharged substrates used for diagnosis are cleaved. The function of the α-chain active site is defective, whereas subunit association, enzyme processing, and the activity of the β-chain are not impaired. Homozygous patients with the B1-mutation show the course of the juvenile disease. However, a late infantile course was seen in compound heterozygotes with a B1 and a null allele.

3.4. Sandhoff disease

The 0-variant of GM2-gangliosidosis was the first gangliosidosis for which the underlying enzymatic defect was identified. It is characterized by storage of negatively charged glycolipids characteristic for Tay–Sachs disease, but also by elevation of uncharged glycolipids such as glycolipid GA2 in the brain and globoside in visceral organs [107]. Clinically, the following forms have been reported:

Infantile Sandhoff disease: In addition to the clinical and pathological manifestations present in Tay–Sachs disease (infantile B-variant), organomegaly and slight bone deformations may also occur.

Juvenile Sandhoff disease: first symptoms such as slurred speech, cerebellar ataxia, and psychomotor retardation appear at

3 to 10 years. Vision is normal, but spasticity increases and mental function deteriorates gradually.

Adult Sandhoff disease: The onset of symptoms is delayed to late adult life. The clinical manifestations in this chronic variant are similar to those of Tay–Sachs disease variants of corresponding ages, with the exception of additional accumulation of uncharged enzyme substrates.

3.5. AB-variant of GM2-gangliosidosis

In this variant, the deficiency of the GM2 activator protein [108] in the presence of normal β-hexosaminidase A, B, and S activity leads to the accumulation of glycolipids GM2 and GA2. The clinical picture resembles that of Tay–Sachs disease with a delayed appearance of symptoms.

Histopathologically, the GM2-gangliosidoses are characterized by the presence of swollen neurons with massive accumulation of storage material in membranous cytoplasmic bodies throughout the central and peripheral nervous system.

The pathogenesis of the GM2-gangliosidoses is not completely understood. Together with other membrane lipids and proteins, ganglioside GM2 accumulates and precipitates within the lysosomes of cells [99], particularly in neuronal cell bodies. Although the storage compounds themselves are neither toxic nor immunogenic, they induce inflammatory responses as demonstrated for glycoconjugates in the murine model of Sandhoff disease [109]. The toxic derivative lysoganglioside GM2 was found in patients with GM2-gangliosidosis [110,111]. It is not clear, however, if this contributes to the pathogenesis of the disease. The induction of misconnections in neuronal dendrites and other cellular processes might be one of the causes of the neurological phenotype. Undegraded storage material might reach, to some extent, cellular membranes like that of the Golgi apparatus or the plasma membrane, and alter membrane composition and function. Bone marrow transplantation (BMT) experiments suggest a complex pathogenetic mechanism that may well involve lytic compounds, for example, in the blood circulation and/or cytokines generated in the brain [112].

The severity of the disease correlates with the residual activity of the defective enzymes [231]. Mutations in the genes encoding the three polypeptide chains have been identified [98].

Naturally occurring animal models of GM2-gangliosidoses have been reported in dogs, cats and pig. In addition, murine models of Tay–Sachs disease, Sandhoff disease and GM2-activator deficiency have been generated using gene-targeting techniques [98]. Unlike human GM2 gangliosidoses, the phenotypes of these murine models differ greatly. These differences among the models result from different ganglioside degradation pathways in mice and humans. In mice, the defective step can be bypassed by the action of a sialidase [113]. An inducible mouse model of the late-onset B-variant has been established [114]. To date, a therapy attenuating the development or reversing the clinical manifestations of the GM2 gangliosidoses is not available, but several therapeutic strategies are studied clinically and in disease models (Section 6). Treatment is restricted to supportive care and appropriate management of the intervening problems.

3.6. Fabry disease

Fabry disease is an inborn deficiency of lysosomal α-galactosidase A. This homodimer of 50 kDa subunits [115] catalyses the lysosomal hydrolysis of globotriaosylceramide. Fabry disease is a panethnic, X-chromosomal-linked inherited disorder with an estimated frequency of 1:117,000 [78] to 1:40,000 birth [115]. Hemizygous males have extensive deposition of globotriaosylceramide in the lysosomes of endothelial, perithelial, and smooth-muscle cells of blood vessels. Many cell types in the heart, kidneys, eyes, cornea, and the autonomous nervous system are also affected.

The first clinical symptoms usually occur during childhood or adolescence. These include severe pain in the extremities, vascular cutaneous lesions, hypohidrosis, and corneal and lenticular opacities. The disease develops to renal, cardiac, and/or cerebral complications, which are the most common causes of death in the 4th or 5th decade of life. A variant of the disease characterized by a milder progression and a primary impairment of the heart muscle has been attributed to enhanced residual activity of more than 5% of normal of the defective enzyme. Heterozygous females have higher residual α-galactosidase A activities, have an attenuated form of this disease, and are usually not as severely affected as hemizygous males. Diagnosis of female patients is facilitated by determination of the α-galactosidase A to β-glucuronidase ratio [116].

The observed symptoms result from accumulating glycolipids in the affected tissues, the blockage of blood vessels, or both simultaneously. For example in the kidney, the lesions are due to glycosphingolipid accumulation in several cell types, but renal blood vessels are progressively and often extensively involved. On the other hand, in the nervous system vascular involvement is the predominant cause of the affection.

About 180 different mutations causing Fabry disease have been identified including partial gene rearrangements, splice-junction defects, and point mutations [115].

An animal model of this disease has been created [117]. Although it shows no clinical symptoms, it allows evaluation of therapeutic strategies, as for treatment using adeno-associated virus mediated therapy, which leads to long-term correction of storage [118]. The treatment of Fabry disease by enzyme replacement therapy using recombinant α-galactosidase A derived from human skin fibroblasts [119] or CHO-cells [120] has been established. A chemical chaperone approach with galactose was successful in a male patient of the cardiac variant of the disease [121], and extensions of this approach using inhibitors have been reported [122–124]. Deoxygalactonojirimycin treatment of knock-in mice that express the (R301Q) variant of α-galactosidase A led to an increase of enzyme activity in the heart and reduction of globotriaosylceramide storage [125].

3.7. Gaucher disease

Gaucher disease is the most common form of the sphingolipidoses [126,127,130]. It is caused by the deficiency

of glucosylceramide-β-glucosidase [128,129] leading to accumulation of glucosylceramide.

Glucosylceramide-β-glucosidase, also called glucocerebrosidase, consists of 497 amino acids and has a molecular weight of about 65 kDa in its glycosylated form [130]. It is a lysosomal enzyme that can associate to membranes. The X-ray structure of this enzyme has been reported [131], also when it was bound to the irreversibly acting inhibitor conduritol β-epoxide [132]. Variant forms of the enzymes with 42 selected single amino acid substitutions have been generated, expressed in insect cells, purified, characterized for kinetic, stability and activator response properties, and mapped onto the crystal structure [133]. The enzyme can be allosterically activated by Sap-C [134,135] and by negatively charged phospholipids [136], of which the lysosomal bis(monoacylglycero)phosphate seems to be of physiological relevance [137].

Three different types of Gaucher disease are distinguished: The attenuated form, Gaucher disease type I, has a nonneuropathic course and is the most frequent form of this disease. It has a frequency of 1: 50 000–200 000 births, but which is higher amongst Ashkenazi Jewish population (1:1000). Life expectancies of these patients range between 6 and 80 years. Gaucher disease type II, the acute form, is a very rare panethnic disease characterized by the involvement of the nervous system with early onset and a life expectancy of less than two years. The subacute or juvenile form, Gaucher disease type III, is an intermediate variant of the other two types, mainly found in the Northern Swedish population. In this case, the neurological symptoms have a later onset and a slower development than in form II; the survival age of the patients is between a few years and four decades. A partial defect in glucosylceramide-β-glucosidase is not associated with visible skin phenotype in humans, but complete enzyme deficiency leads to the "collodion baby" phenotype with a severe impairment of skin function [138].

In all variants, patients may show hepatosplenomegaly, anemia, thrombocytopenia, and bone damage. The severity of these symptoms differ widely, but is inversely correlated with the residual enzyme activity determined in skin fibroblasts of Gaucher patients [139].

Even if the enzyme activity is reduced in all cell types, the phenotype of type 1 of the disease is predominantly manifested in macrophages of the reticuloendothelial system, since these cells have to degrade large amounts of glycolipids derived from the phagocytosis of erythrocytes. Due to the stored material, macrophages acquire a typical morphology, which is characterized by the enlargement of the cell and the occurrence of cytoplasmic linear inclusions. Not only the appearance of these so called "Gaucher cells" in the affected tissues, but also the inflammatory response they induce by the release of cytokines [140], might account for the hypertrophy of the affected organs, for cortical bone loss, and bone marrow disease. Types II and III of the disease that affect the CNS are characterized by a progressive loss of neuronal cells. This might also be caused by accumulation of glucosylsphingosine, which apparently produces neuronal toxicity [141,243] and might be also able to induce inflammatory responses [142].

Together with glucosylceramide accumulation, an elevation of chitotriosidase activity occurs in plasma that can be used for diagnostic purposes [143].

Approximately 200 mutations at the glucosylceramide-β-glucosidase locus have been found in patients with Gaucher disease, four of which account for about 86% of the cases in the Jewish population and for 68% in non-Jewish population. Two cases of Gaucher disease are known, where the cause is the absence of a sphingolipid activator protein, Sap-C [144,225,226]. A certain mutation (N370S) impairs the interaction of variant glucosylceramide-β-glucosidase enzyme with Sap-C [145].

An animal model most resembling the type II form of the disease has been created by targeted disruption of the glucosylceramide-β-glucosidase gene in mice [146]. The animals store glucosylceramide in cells of the reticuloendothelial system and die within 24 h after birth. Recently, additional viable models of Gaucher disease have been developed by introduction of the point mutations N370S, V394L, D409H, or D409V into the mouse glucosylceramide-β-glucosidase locus [147]. Glucosylceramide-β-glucosidase activities were in the range of 2 to 25% of wild type; the homozygous N370S mice were not viable, and the others showed variable pathologies.

Due to the pioneering work of R. O. Brady, enzyme therapy for the attenuated form of Gaucher disease (type I) is available [148]. It consists of the use of glucosylceramide-β-glucosidase purified from placenta or recombinantly expressed, which has been modified in the carbohydrate part to contain targeting information for the mannose receptor on macrophages [149]. After treatment of the patients, a normalization of the blood parameters, as well as a reduced weight of liver and spleen can be observed. Due to CNS involvement, enzyme replacement is less promising for types 2 and 3 of the disease [150]. Also bone marrow transplantations have been accomplished, with variable results [151–153]. A serious problem is the reversal of bone involvement, which has been overcome by peripheral blood stem cell transplantation in the animal model [154].

3.8. Metachromatic leukodystrophy

Metachromatic Leukodystrophy (MLD) is caused by the inherited deficiency of arylsulfatase A (ASA) and the accumulation of sulfatide in several tissues [155]. The storage material accounts for the observed metachromatic staining [156,157]. MLD is a rare disease with an estimated frequency between 1:40 000 and 1:10 0000 in newborns. Arylsulfatase A [157] catalyses the conversion of sulfatide into galactosylceramide and sulfate. For this process, the assistance of Sap-B is required [158]. MLD can be classified into a late infantile, a juvenile, and an adult form.

In the late infantile form, symptoms develop between 6 months and 4 years of age, and death usually occurs about 5 years later. It begins with hypotonia, unsteady gait, and mental regression. Several abilities progressively deteriorate. Common symptoms are loss of speech, blindness, quadriparesis, peripheral neuropathy, and seizures. In a final stage before death, the child is bedridden, in a decerebrate state, and looses all contact with his/her surroundings.

The juvenile form of MLD is characterized by an onset ranging from 4 to 16 years and death usually before 20 years.

The adult form can begin after puberty up to the 6th decade of life and may extend for a few years or for decades. This form is less frequent than the previous two. In both cases, patients show gradual deterioration in school or job performance, with emotional and behavioral disturbances or psychiatric symptoms in the adult form. Other clinical manifestations are gait clumsiness, incontinence, and optic atrophy. During the final stages of this disease, the patient reaches a vegetative state.

The biochemical defect in all forms of MLD is a deficiency in the enzymatic hydrolysis of sulfatides. These sulfated glycolipids occur mainly in the myelin sheaths in the white matter of the brain, in the peripheral nervous system, and in the kidney tissue. The clinical and histopathologic manifestations of MLD are fundamentally caused by a demyelination process. This phenomenon appears to be secondary to sulfatide-induced changes in oligodendrocytes and Schwann cells.

Additionally, lysosulfatide, a cytotoxic sulfatide derivative that occurs in tissues of the patients, seems to play a role in the pathogenesis of this disease [244].

More than 60 different mutations in the ASA gene are associated with the MLD phenotype. Some mutations produce a complete loss of enzyme activity and patients homozygous for this type of allele develop the late infantile form of the disease. On the other hand, low but definite amounts of ASA activity result in milder forms of the disease [159,160,231].

Also the inherited deficiency of Sap-B, the cofactor required for sulfatide cleavage by ASA *in vivo*, leads to a clinical picture similar to MLD caused by ASA deficiency. In this case, the activity of ASA toward soluble, synthetic substrates is normal [161].

A mouse model of MLD has been created [162]. Although it develops some neurologic symptoms, most likely due to sulfatide storage, the mice show no demyelination, which is a hallmark of the human disease. Although it shows only a low extent of neurological and neuropathological changes, this model was of value for characterization of defects in acoustic perception. To date there is no causal therapy for this lethal disease, but enzyme replacement therapy has been successfully evaluated in the animal model: In ASA knockout mice, intravenous ASA injection restored sulfatide metabolism in peripheral tissues and the central nervous system [163]. Also bone marrow transplantation has been applied to patients of the juvenile form [164]. Transplantation of genetically modified stem cells gave promising results in the nervous system of the animal model [165].

In a related disease, multiple sulfatase deficiency known as mucosulfatidosis or Austin disease, the activities of all known sulfatases are strongly reduced [166]. This disease results from a defective posttranslational modification, which is necessary to enable sulfate ester hydrolysis by sulfatases. Cells from these patients show a deficient transformation of a cysteine residue into a formylglycine residue in the active site of different sulfatases [167]. The phenotype of this disease can be described as a combination of symptoms of metachromatic leukodystrophy and a mucopolysaccharidosis. Nine mutations in the sulfatase-modifying factor-I gene (SUMFI) of seven patients have been identified that lead to deficiency of the Cα-formylglycine generating enzyme (FGE) [168].

3.9. Krabbe disease

Krabbe disease or globoid cell leukodystrophy [169,170] is caused by an inherited deficiency of galactosylceramide-β-galactosidase. This membrane-associated enzyme with a molecular weight of about 50 kDa hydrolyzes galactosylceramide to ceramide and galactose [170]. Sap-A and Sap-C are able to stimulate this degradation step *in vivo*. Although there is some storage especially in globoid cells, the enzyme deficiency does not lead to substantial substrate accumulation, probably because of the rapid loss of galactosylceramide synthesizing and accumulating cells. Krabbe disease and MLD are classical myelin diseases.

Clinically, Krabbe disease has two variants: an infantile and a late onset form.

Symptoms of the infantile form usually start between 3 and 6 months of life, and may include irritability or hypersensitivity to external stimuli. Within a short time, severe mental and motor deteriorations occur. Commonly, patients become blind, deaf, flaccid, and hypotonic. The survival of the patients is less than two years.

In the late onset form of globoid cell leukodystrophy, the symptoms can appear at any time after the patients are able to walk; the onset may vary from a few years up to 73 years of age. Common clinical manifestations are psychomotor retardation, blindness, spastic paraparesis, and dementia.

The most characteristic histopathological changes are extensive demyelination, loss of oligodendroglia, astrogliosis, and presence of numerous multinucleated globoid cells. These cells are hematogenous macrophages containing undigested galactosylceramide.

The pathogenesis of this disease can be attributed to a combination of two phenomena: the impaired degradation of galactosylceramide, which leads to globoid cell infiltration, and the accumulation of the cytotoxic derivative galactosylsphingosine (lysogalactosylceramide=psychosine), which causes oligodendroglial cell destruction. Psychosine is another substrate of the deficient enzyme; it accumulates up to toxic, cell-destructive levels. Several mutations in the galactosylceramide-β-galactosidase gene have been identified. A deletion of exons 11–17 is a very frequent mutation and is associated with the infantile form of the disease [171,172].

An authentic mouse model for Krabbe disease is the twitcher mouse with a premature stop codon within the coding sequence of the gene [173]. Another animal model carries a mutation on the galactosylceramide-β-galactosidase gene leading to low enzyme activity [174]. Investigations of the twitcher mouse point to a neuroinflammatory response that might additionally contribute to pathogenesis [175,176]. A double knockout mouse deficient in galactosylceramide-β-galactosidase and the biosynthetic enzyme ceramide galactosyltransferase (cgt) shows shorter lifespan than the (cgt −/−)mice themselves [177].

A mutation within the Sap-A domain of the human [215] and murine [214] Sap-precursor protein causes a deficiency of the mature activator and a phenotype similar to Krabbe disease.

To date, treatment of Krabbe disease is limited to bone marrow transplantation in patients with only minimal neurologic involvement [267,268]. In infantile patients, transplantation of umbilical-cord blood gave promising results, but only when the treatment was started before the onset of symptoms [178]. Recently, peripheral enzyme replacement has been applied to Twitcher mice, which resulted in attenuation of early symptoms and an increase of life span [179].

3.10. Niemann–Pick disease, type A and B

Niemann–Pick disease (NPD), type A and B, is caused by the inherited deficiency of acid sphingomyelinase (ASM) and accumulation of sphingomyelin [184]. Secondarily, sphingomyelin also accumulates in Niemann–Pick disease, type C, in which the products of the NPC-1 or NPC2 genes are deficient [180,181]. In both cases, trafficking of endocytosed cholesterol is altered. Also drug-induced lysosomal storage of sphingomyelin occurs in response to treatment of human patients with tricyclic antidepressants over long time periods. In this case. sphingomyelin accumulates due to the drug-induced degradation of acid-sphingomyelinase [182,183].

Acid sphingomyelinase [184,185] is a glycoprotein with a molecular weight of 70 kDa. Within the lysosomes, it catalyses the degradation of sphingomyelin to ceramide and phosphorylcholine. Its modular structure includes a Sap-like domain and a catalytic domain. Acid sphingomyelinase can be stimulated by lysosomal lipids and sphingolipid activator proteins [186], but this appears not to be necessary *in vivo*.

NPD, type A and B, is a panethnic disease with a higher frequency among Ashkenazi Jews (1:80 for heterozygotes). Type A NPD is a fatal disorder of infancy with a life expectancy of the patients of 2 to 3 years. Affected newborns appear to be normal at birth, but in the first few months of life, symptoms such as hepatosplenomegaly, moderate lymphadenopathy, hypotonia, and muscular weakness appear. Feeding difficulties and splenomegaly lead to a decrease in linear growth and body weight. In later stages of the disease, common manifestations are microcytic anemia, decreased platelet count, osteoporosis, brownish-yellow color of the skin, and cherry-red maculae. Psychomotor retardation becomes evident by 6 months of age and gradually increases over the years. In an advanced state, the patient looses contact with the environment.

Type B NPD is a phenotypically variable disorder with little or no involvement of the nervous system. It is usually diagnosed in childhood, but patients can reach adulthood. Commonly, liver and/or spleen are enlarged and progressive pulmonary infiltration causes the major disease complications in more severely affected patients.

Patients with type B show a higher residual enzyme activity than those with type A [187].

NPD is characterized by the presence of “foam cells” or “Niemann–Pick cells”, although patients with other pathologies may have histologically similar cells. The formation of these histiocytic cells is caused by storage of sphingomyelin and/or other lipids in the monocyte–macrophage system. Storage occurs in spleen and lymph nodes, but also in liver, brain, kidney, and lungs. There is little or no lipid storage in the central nervous system of type B NPD patients.

Sphingolipid-induced alterations in signal transduction [188] might also contribute to NPD pathogenesis. In response to extracellular stimuli, ceramide can be generated by hydrolysis of sphingomyelin, catalyzed by different sphingomyelinases with different topologies. Since in addition to neutral sphingomyelinases, ASM also might play a role in the activation of the so-called “sphingomyelin pathway”, NPD patients may have subtle abnormalities in various signaling pathways and these abnormalities could be exacerbated by stress. There is currently little clinical evidence in support of this hypothesis, however, mice deficient in ASM show a severe impairment in early host defense against *Listeria monocytogenes* [189] and other signalling abnormalities.

Another factor that may contribute to the pathogenesis of the disease is the formation of sphingosylphosphocholine (SPC), a potent mitogen which can induce neurite outgrowth, and which has been shown to accumulate in type A NPD [190].

Different mutations in the ASM gene that cause types A and B NPD have been described. Three mutations, R496L, L302P, and fsP330, account for about 92% of the mutant alleles in Ashkenazi Jewish type A NPD patients. A common mutation in type B patients is the single lesion ΔR608 [184].

Mouse models of NPD have been constructed by using gene-targeting strategies [191,192]. Even if the precise targeting events differed in the two animal strains, they show essentially identical phenotypes. The NPD “knock-out” mice develop features of both types A and B NPD, and are used for evaluation of various therapeutic strategies. To date, there is no specific treatment available for NPD. If performed early in life, BMT has a positive effect on the clinical course of severely affected type B NPD patients [193], but graft versus host disease and other transplant-related complications are common and preclude its use as a routine therapy. The outcome of BMT for the treatment of type A patients is uncertain [194].

3.11. Farber disease

Farber-disease [195] is a rare disease due to the inherited deficiency of lysosomal acid ceramidase and storage of ceramide in the lysosomes. Acid ceramidase [195] is a heterodimeric enzyme composed of an α-subunit of 13 kDa and a β-subunit of 40 kDa [196]. Both subunits are derived from a common 55 kDa precursor that is processed within late endosomes and lysosomes [197]. Acid ceramidase catalyses the degradation of ceramide to sphingosine and a fatty acid in the lysosomes, and requires the presence of Sap-C [198] or Sap-D [199]. It is also able to catalyze the reverse reaction [200]. Point mutations on the acid ceramidase gene in patients of Farber disease have been identified [197,201]; deletion of the gene leads to embryonic lethality at day 8.5 in mice [202].

Usually, the symptoms of Farber disease appear several months after birth, and death occurs within the first years of life.

Patients with milder forms of the disease can reach adulthood. The most characteristic clinical manifestation is the development of painful and progressive joint deformations, subcutaneous nodules (lipogranulomas), and progressive hoarseness. In the granulomas, but also in some organs and tissues, lipid-loaden macrophages are frequent. Apart of skin and joints, liver, spleen, lung, and heart are frequently affected organs. Neuronal accumulation of ceramide and gangliosides has also been reported. A variant form of the disease is present in prosaposin (=Sap-precursor) deficiency [79], where the resulting deficiency also of Sap-D prevents ceramide degradation [199]. The biochemical findings show combined characteristics of Farber disease, and at least four other sphingolipidoses (see Section 3.12.1.).

The important role of ceramide in skin function accounts for the involvement of subcutaneous tissues in Farber disease. The accumulated lysosomal ceramide in Farber disease does apparently not cause apoptosis or another response expected for this signaling substance [203]. The clinical course of this disease correlates with the residual acid ceramidase activity [204]. Therapy of infantile ceramidase deficiency has been attempted with bone marrow transplantation, which improves the peripheral, but not the neurological manifestations [205].

3.12. Deficiency of Saposins (Saps)

The sphingolipid activator proteins Sap-A, -B, -C, and -D are different from the GM2-activator protein. However, the deficiency of the latter protein was the first disease that was attributed to the loss of a sphingolipid activator protein. See the section on the AB-variant of the GM2-gangliosidoses.

3.12.1. Prosaposin

All four saposins are derived from a single protein, the Sap-precursor, or prosaposin, a 70 kDa glycoprotein, which is proteolytically processed to the mature activator proteins (Saps) in the late endosomes and lysosomes [206–208]. Prosaposin is detected mainly in brain, heart, muscle and body fluids, whereas mature Saps are mainly found in liver, lung, kidney and spleen. Until now, two different mutations in few human patients have been reported, a homoallelic mutation of the start codon [209–211] and a homoallelic deletion within the Sap-B domain, which leads to a frame-shift and a premature stop codon [212].

The Sap-precursor deficiency is a fatal infantile storage disorder, characterized by hepatosplenomegaly and severe neurological symptoms. In all human patients, but also in the Sap-precursor knockout mouse [213], there is simultaneous storage of many sphingolipids, including ceramide, glucosylceramide, lactosylceramide, ganglioside GM3, galactosylceramide, sulfatides, digalactosylceramide, and globotriaosylceramide, accompanied by an accumulation of intralysosomal membranes. Prosaposin deficiency can be diagnosed by immunochemical methods, by demonstration of a metabolic defect, or with antisera against more than one Sap protein [238].

3.12.2. Sap-A

Sap-A is required for the degradation of galactosylceramide by galactosylceramide-β-galactosidase in vivo. Mice carrying a mutation in the Sap-A domain of the Sap-precursor protein show accumulation of galactosylceramide and the late-onset form of Krabbe disease [214]. Recently, a human disease has been described that resembles Krabbe disease, but is caused by a singular defect of Sap-A [215].

3.12.3. Sap-B deficiency

Sap-B is the first sphingolipid activator protein that was identified [160]. It is a lipid-transport protein [216] which shows a broader specificity than the GM2-activator. It is able to stimulate the degradation of 20 glycolipids in the presence of human, plant, and bacterial enzymes [217]. In vivo, it mediates the degradation of sulfatide by arylsulfatase A, of globotriaosylceramide and digalactosylceramide as demonstrated in patients with Sap-B deficiency, where these substrates accumulate in the urine [218]. It is also required for the degradation of ganglioside GM3 and lactosylceramide, as reported by studies in cultured human skin fibroblast of Sap-B deficient patients [219]. The crystal structure of unglycosylated human recombinant Sap-B has been solved [220].

The inherited defect of Sap-B leads to an atypical form of metachromatic leukodystrophy, with late infantile or juvenile onset [221]. The disease is characterized by accumulation of sulfatides, digalactosylceramide, and globotriaosylceramide [79]. The clinical findings in Sap-B deficiency are similar to those in metachromatic leukodystrophy. Nine cases with early juvenile and juvenile onset have been reported [79].

3.12.4. Sap-C deficiency

Sap-C was first isolated from spleens of patients of Gaucher disease [222]. It is required for the lysosomal degradation of glucosylceramide by glucosylceramide-β-glucosidase [222] and lends protease-resistance to the enzyme inside the cell [223]. Recently, the solution structure of Sap-C has been solved [224]. In contrast to the mode of action of the GM2-activator and of Sap-B, Sap-C can activate glucosylceramide-β-glucosidase directly [134,135,222]. Sap-C deficiency leads to an unusual juvenile form of Gaucher disease and an accumulation of glucosylceramide mainly in brain, liver and spleen [225,226]. Until now, only two human patients with point mutations in one allele of the Sap-C domain of prosaposin have been diagnosed. The mutations led to the substitution of the same cysteine residue and the subsequent loss of a disulfide bridge in the variant proteins. The clinical findings are similar to those in Gaucher disease, type 3. Until recently, the other allele remained unidentified in the two patients. A recent work [227] demonstrated a Q430X mutation in the Sap-D-domain on the other allele, so that at least one of the patients is compound heterozygote for Sap-C- and prosaposin deficiency.

3.12.5. Sap-D

Sap-D stimulates lysosomal ceramide degradation by acid ceramidase in *in-vitro* [198] and in cultured cells [199]. In Sap-D deficient mice, ceramides, particularly those containing hydroxy fatty acids, accumulate in the kidney and the brain of the animals [228], which, however, suffer also from severe

urinary system defects. Human diseases based on the isolated defect of this cofactor are unknown to date.

4. Pathogenesis of spingolipidoses

Sphingolipidoses display a high degree of phenotypic variability [229]. Onset, development, and symptoms vary widely between different sphingolipidosis, but can also differ drastically within one and the same disease. One primary factor that determines the pathogenesis of the diseases is the cell-type specific pattern of glycosphingolipid expression. Lipid storage in lipidosis patients occurs especially in those cells and organs in which the lipid substrates of the corresponding deficient degrading system are prevalently synthesized or taken up by phagocytosis. Thus, complex gangliosides are predominantly formed in neuronal cells, so that altered catabolism of these glycolipids leads initially to damage of the central nervous system. Ceramide is essential for skin function and contributes to the formation of water permeability barrier. This explains the severe skin-phenotype of patients with a complete loss of glucosylceramide-β-glucosidase activity, which liberates extracellular ceramide from a more water-soluble precursor [138]. Galactosylceramide and sulfatide are characteristic lipids of myelin, so that in both, Krabbe's disease and metachromatic leukodystrophy, the myelin-forming cells are primarily affected. Alternatively, the lipid load of a cell may not be primarily determined by endogenous synthesis of the respective lipid, rather than by uptake. Thus, in Gaucher disease, storage is especially manifest in macrophages, which have large amounts of sphingolipids to degrade after phagocytosis of cells.

Besides the cell type-specific expression of sphingolipids, the second important factor is the residual activity of the defective enzyme in many, although not all LSDs. Onset and severity of the storage disease is partly determined by the residual activity of the gene product in the lysosomes [230,231]. A complete deficiency of a lysosomal enzyme leads to an early onset and a severe course of the disease, whereas only a few percent of residual activity can be sufficient to delay the onset of the disease, cause an attenuated course, and lead to the often misdiagnosed adult forms of the diseases [232]. Although this is valid for most of the LSDs, patients with identical genotype and, accordingly, formal similar residual activity, can undergo different courses of a disease. Apparently, other genetic and epigenetic factors contribute to the expression of a disease in an individual patient.

For some LSDs, so-called enzyme *pseudodeficiencies* have been described. This term refers to individuals who have a substantial reduction of enzyme activity, but do not develop any lysosomal accumulation. Pseudodeficiencies are due to polymorphisms in the genes of the respective enzymes that cause the reduction of enzyme activity. They support the threshold theory [230], according to which only the decrease of the variant enzyme activity below a critical threshold leads to a reduced turnover rate and subsequent accumulation of the substrate. The theory is valid for most sphingolipidoses [81]. It is also the basis for the current therapeutic approaches, since it predicts that slight elevations of enzyme activity can drastically improve the conditions of the patients (see Section 6).

A direct consequence of the metabolic blockage is the accumulation of the corresponding enzyme substrates. The majority of the amphiphilic storage compounds is not excreted by the affected cells, although it is known that lysosomes of some cell types can secrete their contents after fusion with the plasma membrane [233], or undergo calcium ion-regulated exocytosis for plasma membrane repair [234]. Extracellular occurrence of storage material, as that of sulfatide in urine and cerebrospinal fluid in metachromatic leukodystrophy, has been attributed to lysosomal exocytosis [235]. The growing amount of accumulating material might initially lead to a mechanical damage of the cell, and subsequently to apoptosis (e. g. [236,237]). Hypertrophy of affected organs is frequently observed, although the storage material itself contributes only to a small extent to mass increase. For example, an increase in heart weight by 1000 g cannot be explained by the accumulation of an amount of 3.5 g globotriaosylceramide as the storage substance [238]. Therefore, hypertrophy secondary to lysosomal storage is an important factor in pathogenesis.

In addition, the pathogenesis can be influenced by the nature of the storage material. The degradation disorder can be accompanied by formation of bioactive substances. A highly cytotoxic substance, galactosylsphingosine (psychosine) is not sufficiently degraded in cells of patients suffering from Krabbe disease. It leads to the destruction of affected cells, before a significant accumulation can occur [239]. Galactosylsphingosine has been recognized as a ligand of the TDAG8-receptor [240]. Subsequent inhibition of cytokinesis explains the formation of multinuclear globoid cells in the brain of patients of Krabbe disease [241]. In addition, psychosine might also act by uncoupling mitochondrial respiratory chain [242]. Other examples for the formation of toxic substances are glucosylsphingosine in Gaucher disease [243], lysosulfatide in metachromatic leukodystrophy [244], sphingosylphosphorylcholine in Niemann–Pick-disease, type A [245], and lysoglycosphingolipids in the other disorders. Neuronal dysfunction might also develop in response to morphologically active substances [246,247] like ganglioside GM2, which is normally expressed on neurons during neuritogenesis, with subsequent formation of meganeurites and axonal spheroides [73].

Secondary to lipid and glycolipid storage, downstream effects of lysosomal storage contribute to the pathogenesis of these diseases. For example, in patients of Gaucher disease [141], in the mouse model of Sandhoff disease [248], in animal models of other gangliosidoses [249] and in other LSDs [73], inflammatory responses like macrophage activation or cytokine release have been reported. As demonstrated by deletion of the macrophage-inflammatory protein 1α gene in the Sandhoff disease mouse [250], inflammation is an important factor for neuronal death, eventually also via disruption of the blood brain barrier [73]. In addition to macrophage-inflammatory protein 1α, also rab-activation by cholesterol, which secondarily accumulates in most sphingolipid storage diseases, might account for this effect [251]. Other secondary effects, as on lipid trafficking [252], phospholipid metabolism, calcium ion

homeostasis [253,254,258], and mitochondrial function [255] have been observed.

5. Diagnosis

The diagnosis of sphingolipidoses [256] is based on the evaluation of clinical symptoms and characteristic pathological manifestations, analysis of storage compounds, and especially the measurement of enzyme activities of the individual hydrolases. Enzyme sources are serum, leukocytes, cultured skin fibroblasts, amnion cells, chorion villi, or biopsy material. If enzyme activity or stability in serum are low, activity might be determined in lymphocytes or cultured skin fibroblasts. Disease markers like plasma chitotriosidase in Gaucher disease [144] are valuable means for diagnosis and following therapeutic success.

Natural or synthetic substrates with fluorogenic or chromogenic properties are frequently employed [256]. The analysis of substrate accumulation can be demonstrated in cultured cells, degradation capacity can be determined by radiolabelled substrates or catabolic substrate precursors. For example, for Farber disease, radiolabelled ceramide, sulfatide, or sphingomyelin can be used. Also altered pattern of sphingolipid trafficking might be used for diagnostic purposes [252]. The diagnosis of a sphingolipidoses can also be achieved by biosynthetic labelling of the sphingolipids in cells derived from patients and in normal cells. After a long chase period, the substrate whose degradation is impaired will be labelled to a greater extent than in control cells. The advantage of this method is that proteins and lipids are tested in their natural and topological correct environment. Furthermore, the amount of the radioactivity incorporated into the substrate correlates directly with the extent of the enzyme defect. In addition, this method also allows the identification of sphingolipidoses caused by the absence of activator proteins [199].

6. Therapeutic approaches

Many of the discussed disorders remain untreatable and represent a high burden on the patients and their families. The theoretical basis for the therapeutic approaches toward sphingolipidoses is the "threshold theory" [230]. According to this theory, the ratio of substrate influx into the lysosomes and the degradation capacity determines the onset and severity of the diseases. Both parameters can be addressed by therapeutic approaches, especially since the theory predicts that already slight changes in this ratio can improve the condition of the patients.

The objective of most of the causal therapies of sphingolipidoses is the restoration of the defective degradation capacity within the lysosome. The current strategies for causal treatment that are in use or under evaluation [257,258] are enzyme replacement therapy (ERT), cell-mediated therapy (CMT) including heterologous bone marrow transplantation (BMT) and cell-mediated "cross correction", gene therapy, and enzyme-enhancement therapy with chemical chaperones [122,257,259,260]. An additional strategy for the treatment of sphingolipidoses consists in the reduction of substrate influx into the lysosomes. This can be achieved by substrate reduction (substrate deprivation) therapy [261].

6.1. Enzyme replacement therapy (ERT)

The aim of ERT [262,263] is to diminish substrate storage by the exogenous supply of the defective lysosomal enzyme. This was demonstrated for many LSDs in cultured cells, and in part also in animal models, where the proteins are taken up by cells by receptor-mediated endocytosis. The enzymes are generally targeted for uptake by the manose-6-phosphate receptor system, present in nearly all cells, or the mannose receptor, present in cells of the macrophage lineage. To date, ERT of sphingolipidoses is successfully applied to patients suffering from type I of Gaucher disease and Fabry disease [257]. The blood–brain barrier prevents therapeutic enzymes from reaching neural cells and thus limits the applicability of this therapeutic method for treatment of central nervous system diseases.

6.2. Cell-mediated therapy (CMT)

In this case, cells are used as therapeutic agent [264] to replace or compensate the defective cell population with normal equivalents, so that tissue or organ function might be restored, or to release enzymes for uptake by deficient cells ("cross correction"). This can be achieved by bone marrow transplantation (BMT), or by the use of neural progenitor cells as examples of this method. For example, intracerebral transplantation of transduced neural progenitor cells into the brain of the mouse model of Niemann–Pick disease, type A, led to a reversal of storage [265]. Also delivery of functional proteins by axonal transport might be further explored in the future [266].

There is currently evidence that CMT significantly alleviates pathologic manifestations of the CNS in LSDs. There are numerous examples for bone marrow transplantations that led to improvement of therapeutic parameters. This is not necessarily due to cross correction, which has been demonstrated only in few cases, but can also be achieved by immunosuppressive effects. BMT has been applied to human patients, e.g., of Krabbe disease and MLD [267,268]. In animal models of LSDs [269,270], this has led to an improvement of the neurological symptoms and the regression of neuronal injury. More than 500 patients of lysosomal storage disorders have been treated with allogeneic stem cell transplantation with variable success [271]. In animal models, direct implantation of cells and the use of BMT to deliver microglial/brain macrophage precursors have both positive effects.

6.3. Gene therapy

This approach [272] is based on the insertion of a functional copy of the mutated gene into cells, which in turn produce the deficient protein. The gene therapy approach takes advantage of the process of cross correction. The deficient enzyme should be stably over-expressed by a few cells, secreted in high levels, and thus correct the phenotype of adjacent cells. Retro- and lentiviral vectors are currently evaluated [273]. Especially

animal models turned out to be valuable for the evaluation of this concept [274].

At the cell culture level, transduction experiments have been carried out for example with retrovirally-mediated galactosylceramide-β-galactosidase cDNA [275]. A lentiviral vector also corrected β-hexosaminidase deficiency in human Sandhoff fibroblasts [276]. In the mouse model of another LSD, Sly syndrome (mucopolysaccharidosis VII), the visceral pathology was corrected by adenovirus-mediated gene transfer [277,278]. Recent developments include the reversal of storage in the animal model of Tay–Sachs disease by injection of a Herpes simplex vector encoding for the β-hexosaminidase α-subunit into the brain of the animals [279].

Gene therapy is expected to be an effective concept for the treatment of LSDs with CNS involvement. Several approaches have been successfully carried out in the animal model of Tay–Sachs and Sandhoff disease. In the murine model of Sandhoff disease, the intracerebral injection of a recombinant adenoviral vector encoding the β-subunit of β-hexosaminidase A resulted in nearly normal levels of enzymatic activity in the entire brain. The addition of hyperosmotic concentrations of mannitol allowed an enhancement of vector diffusion [280].

In humans, the major difficulty in the employment of this gene therapy approach for treatment of CNS diseases is to efficiently deliver a gene therapy vector to the brain through a systemic route. This, the limited duration of gene expression, and other obstacles remain to be overcome before this treatment can be successfully applied to affected humans. Another technical problem relates to immune response in null patients leading to only transient benefit in experimental models using some vectors.

6.4. Enzyme-enhancement therapy

This recently developed method relies on the use of chemical chaperones [260,263]. These substances can bind to enzymes, which are variant, but not completely defective by certain mutations and have an intact catalytic center. Chemical chaperones stabilize the residual functional conformation, and prevent the premature degradation of such enzymes by the quality control system in the ER [281]. Thereby, they enhance the fraction of the functional variant protein and the degradation capacity in the lysosomes. Substrate analogs and enzyme inhibitors have been used to stabilize the variant proteins defective for example in Fabry disease [122], Gaucher disease [282], GM1 gangliosidosis [95], and GM2 gangliosidosis [283]. Of considerable value for this and the substrate reduction approach are iminosugars of the nojirimycin type (Fig. 5) [284]. Members of this substance class are in clinical use as inhibitors of glycosphingolipid biosynthesis, but have also been investigated as chemical chaperones of various lysosomal glycoside hydrolases.

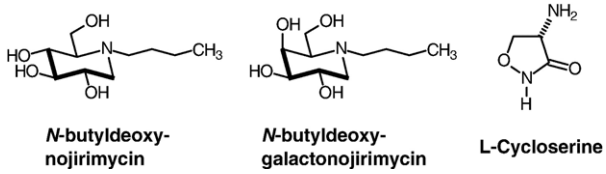

Fig. 5. Structures of *N*-butyldeoxynojirimycin, *N*-butyldeoxygalactonojirimycin, and L-cycloserine.

6.5. Substrate reduction therapy (SRT)

The pathological accumulation of a substance in the lysosome occurs as long as biosynthesis continues. Using inhibitors of sphingolipid biosynthesis, the influx of substrate into the lysosomes may be reduced. The proof of principle of the substrate reduction approach [261,285] has been demonstrated in a genetic model: Sandhoff disease mice were crossbred with mice defective in the biosynthetic enzyme GM2/GD2-synthase [286]. The life span of these animals was much longer, however, they developed a late-onset neurological disease due to the accumulation of oligosaccharides.

Inhibitors of glycosphingolipid biosynthesis [1] are suitable to reduce substrate influx into the lysosomes. For example, the ceramide glucosyltransferase inhibitor *N*-butyldeoxynojirimycin (miglustat, Zavesca®), has been initially investigated in the animal model of Tay–Sachs disease [261]. Its efficacy could be demonstrated in ongoing clinical trials for the treatment of human patients of Gaucher disease, type I [287,288]. Substrate reduction with *N*-butyldeoxynojirimycin and bone marrow transplantation, led to an increased survival of the mouse model of Sandhoff disease, and also treatment with *N*-butyldeoxygalactonojirimycin [289] showed promising results. Addressing the neuroinflammatory response in Sandhoff mice with non-steroidal anti-inflammatory drugs further increased lifetime of the *N*-butyldeoxynojirimycin-treated animals [290]. Inhibition of ceramide glucosyltransferase with *N*-butylnojirimycins is suitable for substrate reduction therapy of diseases that are caused by accumulation of substances derived from glucosylceramide, but not for Niemann–Pick-disease A,B, Krabbe disease, or MLD. In twitcher mice, the model for Krabbe disease, substrate reduction with L-cycloserine (Fig. 5) led to improved conditions and prolonged lifespan [291].

Substrate reduction therapy applied to patients with some residual enzymatic activity or combined with methods, which restore this activity, is expected to be helpful in the treatment of sphingolipidoses.

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