Disorders of Sphingolipid Synthesis, Sphingolipidoses, Niemann-Pick Disease Type C and Neuronal Ceroid Lipofuscinoses

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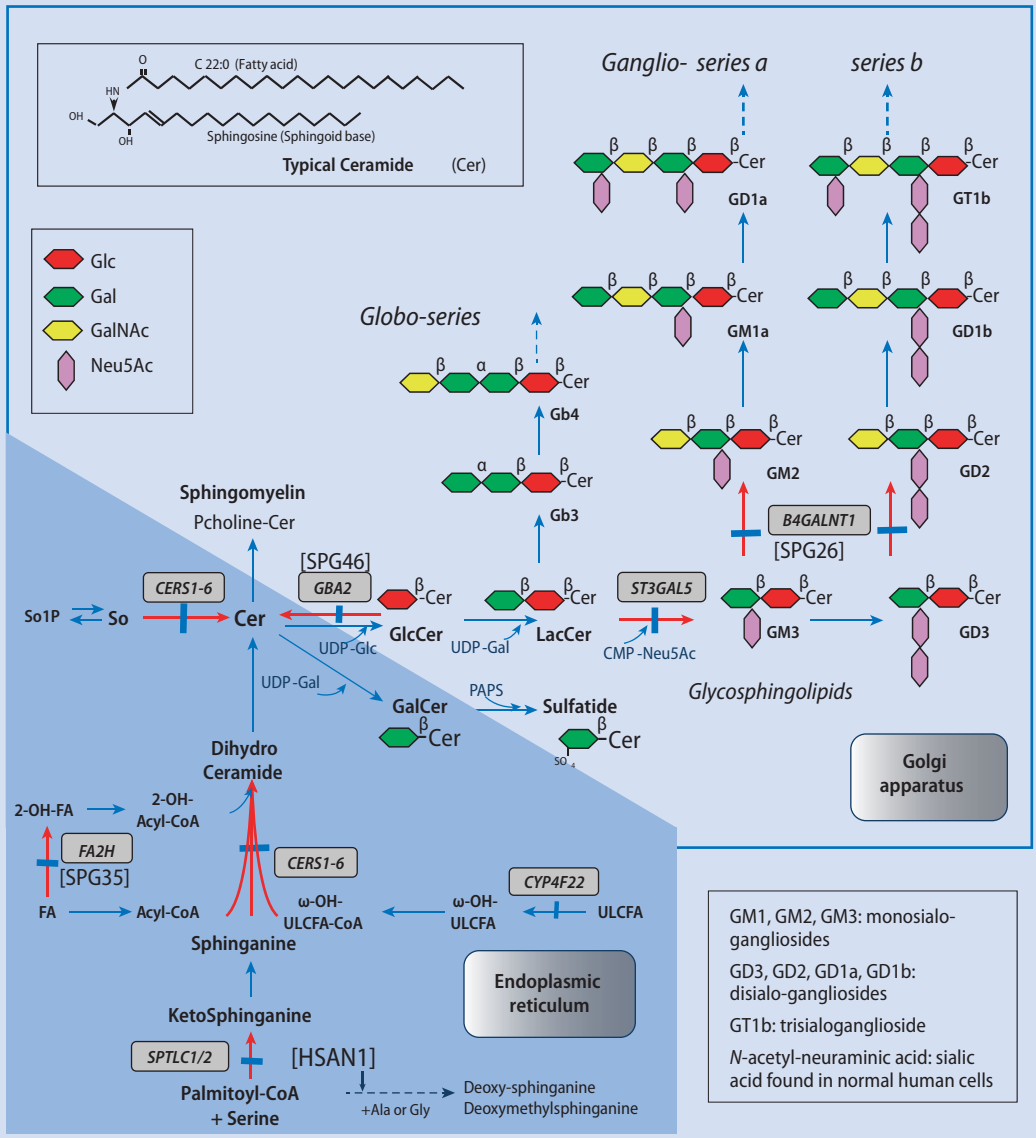


Fig. 38.1 Schematic representation of the structure of the main sphingolipids, and their biosynthetic pathways. Red arrows denote the defective pathways that are discussed in this chapter. The names of genes that are mutated are indicated. *Cer*, ceramide; *FA*, fatty acid; *Gal*, galactose; *GalCer*, galactosylceramide; *GalNAc*, N-acetyl-galactosamine; *Gb3*, globotriaosylceramide; *Gb4*, globotetraosylceramide (globoside); *Glc*, glucose; *GlcCer*, glucosylceramide; *LacCer*, lactosylceramide; *Neu5Ac*, N-acetyl-neuraminic acid; *2-OH-FA*, 2-hydroxy-fatty acid; *ω-OH-ULCFA*, ω-hydroxy ultra-long chain fatty acid; *PAPS*, 3′-phosphoadenosine-5′-phosphosulfate; *Pcholine*, phosphorylcholine; *So*, sphingosine; *So1P*, sphingosine-1-phosphate. Solid bars indicate metabolic blocks

Sphingolipid Structure and Metabolism

Sphingolipids are ubiquitous lipids found in all mammalian cell membranes and in plasma lipoproteins. Many exhibit dual functions, as key structural elements, but also as modulators of numerous biological/physiological functions. Their backbone is a long chain sphingoid base (sphingosine being the prototype) that can be N-acylated by a variety of fatty acids, forming ceramides (Cer) (Fig. 38.1). Depending on the type of hydrophilic head group linked to the 1-OH group of the sphingoid base, two main classes of sphingolipids are distinguished. Phosphosphingolipids contain phosphorylcholine (sphingomyelin), phosphorylethanolamine, or a phosphate group. Glycosphingolipids contain one (glucose or galactose) or several sugar residues, and can be very complex. Sialic acid (N-acetyl-neuraminic acid in humans)

containing glycosphingolipids are named gangliosides. Depending on the precise structure (sugar and linkage) of the oligosaccharide moiety, several glycosphingolipid lineages (ganglio-, globo- etc.) have been defined. ›Cerebroside‹ usually refers to the major myelin lipid galactosylceramide; ›sulfatide‹ to its sulfated derivative. Lysosphingolipids (e.g. psychosine) lack the fatty acid of ceramide. The main sphingolipids are depicted in ◘ Fig. 38.1 and ◘ Fig. 38.2. Sphingolipids are synthesised and degraded in different cellular compartments. A further aspect of sphingolipid homeostasis not discussed here is the recycling or salvage pathway.

Biosynthesis

◘ Fig. 38.1.

The *de novo* synthesis of ceramide occurs in the endoplasmic reticulum and starts by the condensation of serine and palmitoyl-CoA, a reaction catalyzed by serine palmitoyltransferase. The resulting ketosphinganine is reduced to sphinganine prior to N-acylation by ceramide synthases. Distinct fatty acids, including 2-hydroxylated long chain fatty acids and ω-hydroxylated ultra-long chain fatty acids, can be incorporated. Then, dihydroceramide is desaturated to produce ceramide. The sphingosine that is released by the action of ceramidases on the ceramide derived from the degradation of complex sphingolipids can also be N-acylated by ceramide synthases. Subsequent steps of sphingolipid synthesis (except galactosylceramide) occur in the Golgi apparatus, where ceramide is processed to sphingomyelin or to glucosylceramide; stepwise addition of further monosaccharides, catalysed by specific glycosyltransferases, leads to the formation of more complex neutral glycosphingolipids and gangliosides. Sphingolipids are then transported and inserted in various membranes.

Degradation

◘ Fig. 38.2.

After transport by the endolysosomal pathway to the lysosome, sphingolipid degradation proceeds by stepwise hydrolysis by specific acid sphingohydrolases, some of which may need co-factors called sphingolipid activator proteins (also lysosomal) for their *in vivo* action.

Sphingolipidoses are a subgroup of lysosomal storage disorders in which sphingolipids accumulate in one or several organs as the result of a primary deficiency in enzymes or activator proteins. Niemann-Pick type C disease is currently considered as a lipid trafficking disorder, resulting in lysosomal accumulation of cholesterol and sphingolipids. This chapter also describes neuronal ceroid lipofuscinoses, now recognised as another subgroup of lysosomal storage diseases. Additionally, inherited defects of sphingolipid biosynthesis are presented (▶ Sphingolipid Structure and Metabolism).

38.1 Disorders of Sphingolipid Synthesis

Most of the genes encoding the enzymes, transporters and activators operating in the sphingolipid synthesis pathway have been characterised, and an increasing number of monogenic defects affecting some steps of the biosynthesis of sphingolipids have been delineated in recent years (◘ Table 38.1). A majority of disorders were first described as a component of a genetically heterogeneous clinical syndrome -e.g., hereditary sensory and autonomic neuropathies (HSAN), autosomal recessive hereditary spastic paraplegias… – before the function of the protein encoded by the mutated gene was recognised. Mechanisms underlying the pathophysiology of most sphingolipid synthesis disorders are still enigmatic. With the exception of HSAN1, the reported mutations result in a loss of function of the corresponding enzyme. Alterations in the sphingolipid profile of the diseased tissues have not been described in all conditions. In general, it is still unknown whether tissue dysfunction and symptoms are due to the lack (or insufficient production) of one or more sphingolipid species, and/or accumulation of a precursor molecule or a potentially toxic metabolite.

Regarding genetic transmission, with the exception of HSAN1 due to a defect in serine palmitoyltransferase 1 or 2, and possibly the defect in ceramide synthase 2, enzymatic deficiencies of sphingolipid synthesis are inherited as autosomal recessive traits. So far, their diagnosis relies on DNA analysis although biochemical testing in plasma is possible for detection of HSAN1. There is currently no effective specific therapy for this type of IEMs. Most of these conditions remain extremely rare. Their clinical spectrum is broadening with description of new cases and the field is likely to quickly evolve in the near future. For these reasons, and since comprehensive reviews on the subject with exhaustive referencing have been published very recently [1][2], only a brief outline of each disorder will be given in this chapter.

38.1.1 Serine Palmitoyltransferase (Subunit 1 or 2) Deficiency and HSAN1

A defect in the very first step of sphingolipid biosynthesis is the major cause underlying the dominant hereditary sensory and autonomic neuropathy (HSAN1). Other (unrelated) genes that have been linked to HSAN1 are *ATL1*, *RAB7A* and *DNMT1* [1]. This peripheral neuropathy is characterised by a late onset (between the 2nd and 4th decade), a slow disease progression, and primarily sensory deficits (loss of pain and temperature sensation spreading from the distal limbs). Painless ulcerations in the lower limbs are quite frequent, as well as spontaneous lancinating pain attacks. Hypohidrosis is also seen. Some patients exhibit a more severe phenotype, starting in early childhood, with motor involvement, global hypotrophy, and developmental retardation [1].

Table 38.1 Sphingolipid biosynthesis disorders

Enzyme	Gene	Metabolic disturbance	Main clinical features
Disorders with primarily nervous system involvement			
Serine palmitoyltransferase, subunit 1 or 2	*SPTLC1* or *SPTLC2*	Accumulation of 1-deoxysphingolipids (in plasma)	[HSAN1] - Peripheral sensory neuropathy, distal sensory loss, ulcerative mutilations
Ceramide synthase 1	*CERS1*	Possibly decreased C18-ceramide levels (in cultured cells)	Progressive myoclonic epilepsy and cognitive decline
Ceramide synthase 2	*CERS2*	Possibly decreased very-long chain ceramide levels (in cultured cells)	Progressive myoclonic epilepsy
Fatty acid 2-hydroxylase	*FA2H*	Possibly decreased hydroxylated sphingomyelin levels (in cultured cells)	Spastic paraplegia [SPG35], dystonia, dysarthria, ataxia
Non-lysosomal β-glucosidase	*GBA2*	Unknown	Spastic paraplegia [SPG46], cerebellar ataxia
GM3 synthase	*ST3GAL5* (*SIAT9*)	Lack of GM3, GD3 and higher gangliosides, and increased lactosylceramide and Gb4 levels (in plasma and cultured cells)	[Amish infantile epilepsy] Epilepsy, intellectual disability, »salt and pepper« syndrome
GM2/GD2 synthase	*B4GALNT1*	Decreased GM2 and increased GM3 levels (in cultured cells)	Spastic paraplegia [SPG26], ataxia
Disorders with primarily skin involvement			
Ceramide synthase 3	*CERS3*	Lack of ceramides with very-long chain fatty acids (in cultured cells)	Ichthyosis
(Ultra-long chain) fatty acid ω-hydroxylase	*CYP4F22*	Decreased ultra-long acylceramide levels (in skin and cultured cells)	Ichthyosis

Current evidence related to the metabolic derangement points to the accumulation of abnormal sphingoid bases as the main pathogenic mechanism. Specific mutations of *SPTLC1* or *SPTLC2* encoding subunits 1 or 2 of serine palmitoyltransferase, the first and rate-limiting step in the *de novo* synthesis of sphingolipids, alter its substrate specificity. Instead of using L-serine as a substrate (Fig. 38.1), the mutant enzyme preferentially uses L-alanine or L-glycine. The resulting 1-deoxysphinganine and 1-deoxymethyl-sphinganine, and 1-deoxyceramides (or some other derivatives), which cannot be converted to complex sphingolipids, appear to account for the observed neurotoxicity. Of note is the fact that only several missense mutations in the *SPTLC1* or *SPTLC2* genes cause the autosomal dominant disorder HSAN1. Substitution of Ser331 in the subunit 1 of serine palmitoyltransferase seems to result in an early-onset and more severe phenotype.

When a hereditary sensory neuropathy is suspected, elevated plasma levels of 1-deoxy-sphinganine and 1-deoxymethyl-sphinganine, as determined by liquid chromatography coupled to mass spectrometry, provide a strong biochemical argument in favour of a SPTLC1/2 defect. Moreover, plasma 1-deoxy-sphingolipid levels seem to correlate with disease severity.

There is currently no effective specific therapy. However, a 10-week pilot study on patients affected with HSAN1 showed that, like in a mouse model for this disease, L-serine supplementation (200 or 400 mg/kg/day) could reduce the plasma levels of 1-deoxysphingolipids [3]. Whether such a supplementation can ameliorate the sensory deficits requires further investigation.

38.1.2 Defects in Ceramide Synthases 1 and 2 and Myoclonic Epilepsy

Six human ceramide synthases, encoded by *CERS* genes, have been characterised. They display distinct tissue-specificities as well as acyl-CoA substrate specificities, which can explain the neurological (CERS1 and 2) or dermatologic (CERS3, see 38.1.7) expression in case of a defect in one of them.

Very recently, a homozygous missense mutation in *CERS1* has been identified in 4 siblings of an Algerian family showing progressive myoclonic epilepsy and cognitive decline/dementia. The mutation was associated with decreased C18-ceramides levels in cultured fibroblasts. It has also been proposed that progressive myoclonic epilepsy since age 10 in an adult patient (associated with ataxia, dysarthria and photosensitivity) was due to a heterozygous deletion of *CERS2* together with decreased very-long chain ceramides in fibroblasts [1].

38.1.3 Fatty Acid 2-Hydroxylase Deficiency (SPG35/FAHN)

Mutations in *FA2H* encoding fatty acid 2-hydroxylase result in a complex hereditary spastic paraplegia, SPG35, also called fatty acid hydroxylase-associated neurodegeneration (FAHN). To date, 38 patients have been reported, with varied ethnicity. Most patients present in childhood and develop slowly progressive lower and then upper limb spasticity, dysarthria and mild cognitive decline. Dystonia is another common neurologic feature. MRI shows signs of leukodystrophy and diffuse cortical and pontocerebellar atrophy. Neurodegeneration with brain iron accumulation (NBIA), mostly located in the globus pallidus (T2 hypodensity, but no »eye of the tiger« sign), can occur, although not in all patients. The clinical spectrum of SPG35 is widening, with later onset patients and more clinical variability.

The underlying abnormality is likely the insufficient production of 2-hydroxy-galactosphingolipids. Indeed, 2-hydroxylated long chain and very-long chain fatty acids are essentially found in galactosylceramides and sulfatides from myelin, and their proportion relative to non-hydroxylated fatty acids is known to increase with brain development and myelin maturation. Not unexpectedly, in *Fa2h*-deficient mice, brain galactosylceramides were found to contain almost exclusively non-hydroxylated fatty acids.

38.1.4 GM3 Synthase Deficiency and Amish Epilepsy Syndrome

The deficiency of GM3 synthase resulting from *ST3GAL5 (SIAT9)* mutations causes an autosomal recessive infantile-onset symptomatic epilepsy, also called Amish epilepsy syndrome. During the first 3 months of life, affected children show irritability and failure to thrive. Then, within the first year of life, generalized tonic-clonic seizures as well as other seizure types develop, along with a profound developmental stagnation and regression. In some patients, brain MRI shows occipital white matter abnormalities and atrophy in the visual cortex. The severity of the disease varies significantly, some patients suffering from visual loss and deafness. A majority of patients exhibit hyperpigmented maculae on the dorsal part of hands and feet, but also in other locations. Some patients also show patches of skin depigmentation. These skin changes are not associated with the severity of the neurologic disease. The combination of hyper and hypo-pigmented skin maculae, facial dysmorphism, scoliosis, intellectual disability, seizures, choreoathetosis, and spasticity has been described under the term »salt and pepper« syndrome. Associated biochemical features in plasma and cultured cells are the lack of GM3, GD3 and higher gangliosides, and increased lactosylceramide and Gb4 levels.

38.1.5 GM2/GD2 Synthase Deficiency (SPG26)

Mutations of *B4GALNT1* resulting in a defect of GM2/GD2 synthase are associated with SPG26, a slowly progressive complex hereditary spastic paraplegia with mild to moderate cognitive impairment. Ten multiplex families from various ethnic origins have so far been described. The clinical picture is a progressive weakness, with spastic gait and lower limb spasticity. EMG shows an axonal sensorimotor neuropathy in many patients. The disease can be accompanied by cerebellar symptoms, dysarthria, and dysphagia [4]. Studies in cultured fibroblasts of patients have shown decreased GM2 levels with an increase of its precursor, GM3.

38.1.6 Nonlysosomal β-Glucosidase GBA2 Deficiency: SPG46 and Ataxia

GBA2 is a membrane-associated protein localised at the endoplasmic reticulum and Golgi, most likely facing the cytosol. This enzyme can hydrolyse glucosylceramide to ceramide and glucose. While acting on the same substrate but in a different subcellular location, GBA2 is very distinct from the lysosomal acid β-glucosidase GBA1 deficient in Gaucher disease (▶ Section 38.2.1). The formed ceramide re-enters the biosynthetic pathway (◘ Fig. 38.1), or could play a role as a bioactive lipid in case of excessive formation.

Since 2013, several studies have shown that mutations in *GBA2* should be added to the heterogeneous group of ARCA (autosomal recessive cerebellar ataxias), and also underlie the hereditary (complex) spastic paraplegia locus SPG46. Most patients with GBA2 deficiency develop since childhood a marked spasticity in lower extremities with progressive gait disturbances and later, ataxia and other cerebellar signs. Variable additional symptoms have been reported, such as hearing loss or cognitive impairment. Some patients presented testicular hypotrophy associated with spermatozoid head abnormalities [1]. Besides DNA sequencing, diagnosis can be achieved by determination of enzyme activity using a specific method.

Potential interactions between GBA1 and GBA2 may play a role in Gaucher disease. The paradoxical clinical amelioration reported in mouse models of Gaucher and Niemann-Pick C (▶ Section 38.3) diseases after GBA2 inhibition remains an intriguing observation [5][6].

38.1.7 Ceramide Synthase 3 and Ultra-Long Chain Fatty Acid ω-Hydroxylase (CYP4F22) Deficiencies: Autosomal Recessive Congenital Ichthyosis (ARCI)

Autosomal recessive congenital ichthyosis (ARCI) represents a heterogeneous group of disorders of epidermal cornification, in which 9 causative genes have to date been identified. Two of those, *CERS3* and *CYP4F22* encode proteins involved in ceramide synthesis (◘ Fig. 38.1). Specific ceramides are

particularly abundant in the stratum corneum of the skin, where they play a crucial role in maintaining skin barrier homeostasis, preventing water loss and protecting against microbial infections. These ceramides may in particular contain α- or ω-hydroxylated fatty acids and ultra-long chain fatty acids (ULCFAs) (C26 or longer). Acylceramides are unique, very hydrophobic ceramide species present in the epidermis, which contain C28-C36 ω-hydroxylated ULCFAs and are further esterified with linoleic acid.

Ceramide synthase 3 (*CERS3*), which is markedly expressed in the skin, generates epidermis-specific ceramides by N-acylating dihydrosphingosine with ULCFA-CoAs (and likely ω-hydroxylated ULCFA-CoAs). Indeed, functional analysis of a skin sample and *in vitro* differentiated keratinocytes from a patient with a *CERS3* missense mutation severely affecting enzyme activity demonstrated an impairment in the synthesis of ceramides with non-hydroxylated and ω-hydroxylated ULCFA moieties, disturbing epidermal differentiation and leading to premature keratinisation. *CYP4F22* encodes the fatty acid ω-hydroxylase required for acylceramide synthesis, using ULCFAs as substrates [7].

Acylceramides in the stratum corneum have been shown to play a key role in the formation and stabilization of cornified envelopes through covalent binding to corneocyte proteins, and ultimately in skin permeability barrier. It is therefore logical that defective synthesis of these lipids will manifest as severe skin disorders. For the above two defects, the ARCI clinical phenotype has been quite variable in different patients, often including lamellar ichthyosis and palmo-plantar hyperlinearity, but also in some cases collodion membrane at birth (that may be self-improving) and more or less severe congenital ichthyosiform erythroderma. For ichthyotic manifestations due to these defects, topical application of some specific ceramides could be envisioned.

38.1.8 Mutations in Ceramide Kinase-Like (CERKL) Gene and Retinal Dystrophy

Mutations in *CERKL* have been associated with a group of inherited retinal dystrophies presenting as retinitis pigmentosa or cone rod dystrophy. The name ceramide kinase-like was given because of 29% identity and 50% similarity with the human ceramide kinase that converts ceramide into ceramide 1-phosphate, but neither the substrate nor the function of the CERKL protein are still known. At the current stage of knowledge, this disorder thus does not belong to sphingolipid synthesis disorders. It has been listed here by default due to its name, as it cannot yet be classified from a metabolic viewpoint (see [1] for more details).

38.1.9 Alkaline Ceramidase 3 (ACER3) Deficiency: Infantile Leukodystrophy

Similarly, deficiency of ACER3 is reported here because it is not a lysosomal storage disease; whether it represents a true sphingolipid synthesis or a remodelling defect remains to be determined. A homozygous missense mutation in *ACER3*, coding for alkaline ceramidase 3 (localised to both the Golgi complex and the ER), has recently been described in two siblings with leukodystrophy. They presented with neurological regression at 6–13 months of age, truncal hypotonia, appendicular spasticity, dystonia, optic disc pallor, peripheral neuropathy and neurogenic bladder. The *ACER3* mutation was associated with undetectable ACER3 catalytic activity towards natural and synthetic ACER3-specific substrates, and an accumulation in plasma of ACER3 substrates, C18:1- and C20:1-ceramides and dihydroceramides, as well as some complex sphingolipids, including monohexosylceramides and lactosylceramides [8].

38.2 Sphingolipidoses

Sphingolipidoses are a subgroup of lysosomal storage disorders in which sphingolipids accumulate in one or several organs as the result of a primary deficiency in enzymes or activator proteins involved in their degradative pathway. Except for Fabry disease (X-linked recessive), the mode of inheritance is autosomal recessive. The clinical presentation and course of the classic forms are often typical. With the help of relevant procedures (e.g. imaging, neurophysiology, ophthalmology), examination of the patient and perusal of the disease history (especially age at and type of first symptom) should lead to a provisional diagnosis and oriented laboratory tests. Late-onset forms, often less typical, have been overlooked in the past. No global biochemical screening procedure is available to date. In most sphingolipidoses the diagnosis is easily made by demonstration of the enzymatic defect, generally expressed in most cells and tissues (leukocytes represent the most widely used enzyme source, followed by dried blood spots). In a few instances, other biochemical tests and/or a molecular genetic assessment are necessary. Efforts should be made to genotype all patients. Specific therapies are well established for non-neuronopathic Gaucher and Fabry diseases, and under clinical trial for Niemann-Pick B. In spite of active research and ongoing preclinical and clinical trials, progress towards therapy of the neurological forms remains limited.

38.2.1 Gaucher Disease

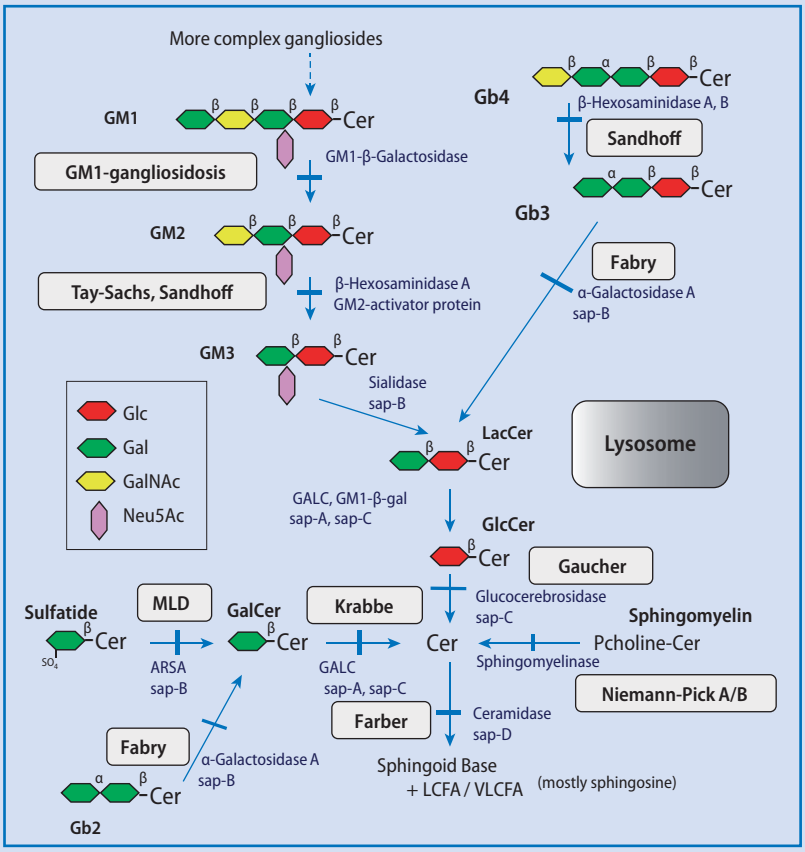
Clinical Presentation

Historically, three clinical phenotypes are recognised, but the full disease spectrum is a continuum. All types are panethnic, but type 1 has a particularly high prevalence in the Ashkenazi Jewish population (carrier frequency 1:13). The overall incidence is about 1 in 40,000 to 1 in 50,000 live births [9].

Type 1 It is defined by the lack of neurological symptoms, and accounts for about 90% of all cases. It can present at any age, but manifests in childhood in more than half of patients [10]. There is a wide variability in the pattern and severity of the

Sphingolipid Degradation and Sphingolipidoses

Only the main sphingolipids implicated in sphingolipidoses are depicted in ◘ Fig. 38.2. The scheme illustrates their degradation by stepwise hydrolysis, and shows enzymatic blocks leading to a disease. It also indicates which sphingohydrolase or sphingolipid activator protein (► Section 38.2.4 and ► Section 38.2.9) is implicated, as well as the name commonly used to designate the various disorders.



◘ **Fig. 38.2 Sphingolipid degradation.** *ARSA*, arylsulfatase A; *GALC*, galactocerebrosidase; *GalCer*, galactosylceramide (or galactocerebroside); *GalNAc*, N-acetyl-galactosamine; *Gb2*, galactobiosylceramide; *Gb3*, globotriaosylceramide; *Gb4*, globotetraosylceramide (globoside); *GlcCer*, glucosylceramide (or glucocerebroside); *GM1*, GM1 ganglioside; *GM2*, GM2 ganglioside; *GM3*, GM3 ganglioside; *LacCer*, lactosylceramide; *LCFA*, long chain fatty acids; *MLD*, metachromatic leukodystrophy; *Neu5Ac*, N-acetyl-neuraminic acid (sialic acid); *Pcholine*, phosphorylcholine; *sap*, saposin. *VLCFA*, very long chain fatty acids. Enzyme defects are indicated by solid bars across the arrows

symptoms, from extremely handicapping to asymptomatic forms, with most symptomatic patients having visceral, haematological and (more frequently in adults) skeletal disease [11]. Children often show severe splenomegaly, generally associated with hepatomegaly. The degree of visceromegaly is highly variable, in both children and adults. This may lead to anaemia, thrombocytopenia and, thus, a bleeding tendency. Leukopenia is less frequent. Children may show delayed growth and menarche. Subcapsular splenic infarctions may cause attacks of acute abdominal pain and medullary infarction of long bones, excruciating pain referred to as bone crises. Essentially in adult patients, bone involvement represents a major cause of morbidity. Aseptic necrosis of the femoral head and spontaneous fractures due to osteopenia are other common complications. Lung involvement with diffuse infiltration may occur. In adults, pulmonary hypertension has been described in rare, usually splenectomised, patients. Co-morbidities with close association to Gaucher disease have been identified, particularly non-Hodgkin's B-cell lymphoma and multiple myeloma, and Parkinson's disease [12][13][14]. Peripheral polyneuropathy was also reported more frequently than in a control population.

Type 2 (acute neuronopathic Gaucher disease) Classically, patients present early in infancy with brain stem dysfunction and pyramidal signs. Retroflexion of the neck, opisthotonus, feeding difficulties and squint are major early signs, apnoeas appear later, and trismus and stridor are less frequent. Splenomegaly is constant but may not be present initially [15][16][17]. Exceptional cases have manifested as haemophagocytic lymphohistiocytosis. The downhill clinical course is rapid, with pronounced spasticity, failure to thrive and cachexia, and few of these patients survive beyond the age of 2 years. Some other patients show strabismus, paucity of facial movements, less sign or none at all of pyramidal involvement, irritability or cognitive impairment and a slower course (some survive up to 5 years) [15][16][17].

The perinatal lethal form is associated with hepatosplenomegaly, pancytopenia and skin changes. Many of these cases are associated with hydrops fetalis, and some have been described as ›collodion babies‹. Arthrogryposis is seen in 40% of cases [17][18][19].

Type 3 (subacute or chronic neuronopathic Gaucher disease) This type is heterogeneous. The mean age at onset is 5 years (but between 5 months and 46 years), with a mean age of neurological onset around 8 years. The most common form consists in severe systemic involvement and supranuclear saccadic horizontal gaze palsy, with or without developmental delay, hearing impairment and other brain stem deficits [10][20][21]. The second most common phenotype shows a relatively mild systemic disease but progressive myoclonic encephalopathy, with seizures, dementia and death. There are also patients with severe systemic involvement and supranuclear gaze palsy who develop a progressive myoclonic encephalopathy [10][20][21]. Brain stem auditory evoked response (BAER) testing may reveal abnormal wave forms (III and IV). A particular presentation with cardiac involvement (heart valve and aortic calcification), supranuclear gaze palsy, mild hepatosplenomegaly and bone disease, has been associated with homozygosity for the D409H mutation. In neurological Gaucher disease, extrapyramidal involvement has also been observed.

Metabolic Derangement

The primary metabolic defect resides in a block of the lysosomal degradation of (β-)glucosylceramide (glucocerebroside) and glucosylsphingosine. In the vast majority of cases this is due to the deficient activity of acid β-glucosidase (glucocerebrosidase, glucosylceramidase) (Fig. 38.2). Exceedingly rare cases, presenting as type 3 or 1 (reviewed in [22]) are due to a deficiency of the saposin sap-C, which is required for the in vivo hydrolysis of glucosylceramide. Glucosylceramide accumulates massively in liver and spleen of patients in all types. Although elevated in cerebral grey matter of type 2 and type 3 patients, its concentration in brain remains low. Glucosylsphingosine also accumulates (but not in brain of type 1 patients). Pathophysiology of the disease is poorly understood [9]. Cytotoxicity due to glucosylsphingosine, disruption of cellular Ca^{2+} homeostasis, as well as inflammatory responses appear to be involved.

Genetics

The disease (except for sap-C deficiency) is caused by mutations in *GBA1* [9]. More than 430 mutations are known. N370S (now c.1226A>G; p.Asn409Ser), the most common one in Ashkenazim, is also very frequent in Caucasian populations. The finding of one single N370S is predictive of a non-neuronopathic phenotype. The severity can vary widely in Gaucher patients with the same genotype, including N370S homozygotes [23]. The second most frequent mutation, L444P (now c.1448T>C; p.Leu483Pro), first described in Norbottnian type 3, is more frequently associated with types 2 and 3. Complex alleles due to genetic rearrangements are more often associated with severe forms, including perinatal lethal forms [19]. A number of genotype-phenotype correlations have been made [10].

Diagnostic Tests

Bone marrow examination (not mandatory) may have revealed Gaucher cells (multinucleated reticuloendothelial cells with a vacuolated cytoplasm, with a »wrinkled tissue paper« appearance). Signs of haemophagocytosis with lymphohistiocytosis have been observed in exceptional cases. Several plasma biomarkers, e.g. chitotriosidase, the chemokine CCL18/PARK, and glucosylsphingosine [24] are typically very elevated, but they are above all used to monitor treated patients. The assay of glucocerebrosidase activity in peripheral blood lymphocytes/ leukocytes or dried blood spots (using fluorogenic or short-chain glucosylceramide substrates) constitutes the primary diagnostic test. Cultured cells have a much higher activity. DNA testing, complicated by the existence of a highly homologous pseudogene, is required for carrier detection and improves diagnostic accuracy for patients with high residual enzyme activity. In sap-C deficiency, glucocerebrosidase activity is normal; the findings of Gaucher cells and elevated levels of the above biomarkers should lead to *PSAP* gene sequencing [25]. In all cases, lipid studies in liver and/or spleen would reveal pathognomonic glucosylceramide storage.

Treatment and Prognosis

Two approaches are currently available for the specific treatment of type 1 (and to some extent type 3 [20]) patients: enzyme replacement therapy (ERT) and substrate reduction therapy (SRT) (see [26] for review). Splenectomy enhances the risk of progression of the disease at other sites, especially bone and lung, and should be avoided. Pregnancy is not contraindicated in untreated patients, but bleeding may become critical before and after birth, and there is now a good experience of ERT throughout pregnancy. Enzyme therapy, conducted with slow infusions of a recombinant enzyme exposing mannose groups (optimal uptake by macrophages), has largely proved safe and effective [9][27]. Imiglucerase has been used worldwide for over 20 years, and two other products, velaglucerase alfa [28][29] and taliglucerase alfa [30] have come onto the market. The natural history of type 1 can be dramatically improved. ERT prevents progressive manifestations and ameliorates Gaucher disease-associated anaemia, thrombocytopenia, organomegaly, bone pain and bone crises. However, the enzyme does not

cross the blood-brain barrier, and this treatment has no effect on the neurological manifestations of type 2. While ERT aims at restoring the degradation rate of the accumulated substrate, SRT tends to reduce the cell burden by slowing down the rate of synthesis of the substrate to a level where it can be slowly cleared by a deficient enzyme with some residual activity. This may be achieved by small molecules that can be administered orally. Two inhibitors of glucosylceramide synthase are currently approved for treatment of Gaucher type 1: the iminosugar miglustat [31][32] and, more recently, eliglustat [33]. Although pharmacological chaperones constitute an attractive approach, the only clinical trial so far did not reach phase III. Other potential strategies are still very experimental [26].

38.2.2 Acid Sphingomyelinase-Deficient Niemann-Pick Disease (Type A, Type B and Intermediate Forms)

Since the early 1980s, the heterogeneous group of »Niemann-Pick disease« has been divided in two separate entities: ›acid sphingomyelinase-deficient Niemann-Pick disease‹ (ASM-deficient NPD) [34], and Niemann-Pick disease type C (▶ below).

Clinical Presentation

ASM deficiencies have historically been categorised into a severe, acute neuronopathic form, or type A, and a non-neuronopathic form, or type B, but there appears to be a continuum ranging from mild to severe type B, and then from late-onset neurological forms toward severe classic type A. Type A has its highest prevalence in Ashkenazim and is rare in other ethnic groups. Type B does not have an Ashkenazi Jewish predilection, and appears to be more frequent in southern Europe, North Africa, Turkey and the Arabian Peninsula than in northern Europe.

Classic Niemann-Pick disease type A The neonatal period is usually normal, with vomiting or diarrhoea, or both, appearing in the first weeks of life. Failure to thrive often motivates the first consultation, leading to the discovery of a prominent and progressive hepatosplenomegaly and lymphadenopathy, in most cases before 3-4 months of age and sometimes earlier. Hypotrophy is observed in 70% of the cases [35]. Neurological examination is essentially normal until the age of 5-10 months, when the child shows hypotonia, progressive loss of acquired motor skills, lack of interest in the surroundings and reduction of spontaneous movements. Psychomotor retardation may at first be overlooked owing to the poor general condition. Initial axial hypotonia is later combined with bilateral pyramidal signs. A decrease of nerve conduction velocities is generally present. A cherry-red spot in the retina is a typical feature, but is often not present until an advanced stage. Severe cachexia is common. Loss of motor function and intellectual deterioration continue to the point where patients become spastic and rigid. Seizures are rare. Brownish-yellow discoloration and xanthomas may be detected in the skin. Death usually occurs between 1.5 and 3 years. Cases with a milder systemic involvement, slightly protracted onset of neurological symptoms and slower course are also seen [35].

Type B Type B is a chronic, non-neuronopathic disease, with a very variable degree of systemic involvement. Most typically, the presenting sign is splenomegaly or hepatosplenomegaly in late infancy or childhood [36], but discovery may occur at any age from birth until late adulthood. Bruising and epistaxis are frequent. Hypersplenism occurs in a small proportion of patients. Splenectomy, seldom necessary, should be avoided. The most constant associated signs are radiographic abnormalities of the lung (diffuse, reticulonodular infiltrations) and interstitial lung disease with variable impairment of pulmonary function [37]. In adults with a long follow-up, pulmonary involvement is often the main complaint, ranging from dyspnoea on exertion (frequent) to oxygen dependency. In children, retarded body growth is a common finding between the ages of 6 and 16 years. Skeletal age and puberty are often delayed [36]. Alterations of liver function are in general mild, but a few cases have been described with liver cirrhosis and liver failure. Hypercholesterolaemia with markedly decreased HDL cholesterol is common even in children. Other features associated with the disease are joint/limb pain, bruising, headache, abdominal pain and diarrhoea. True type B patients do not have neurological involvement and are intellectually intact, but ophthalmoscopic examination may reveal a retinal macular halo or cherry red maculae [36]. Although there are severe forms, the most frequent clinical phenotype is that of a moderately serious disorder compatible with an essentially normal life-span. In a longitudinal study the disease was characterised by hepatosplenomegaly with progressive hypersplenism, worsening atherogenic lipid profile, gradual deterioration in pulmonary function and stable liver dysfunction.

Intermediate forms of ASM-deficient NPD This is a heterogeneous category. Some patients are closer to type A with a late infantile, juvenile or adult neurological onset and a slowly progressive disease that may include cerebellar ataxia, extrapyramidal involvement or psychiatric disorders [38][39]. Some others are closer to type B, with minimal nervous system involvement (often peripheral neuropathy) and/or mild mental retardation [40].

Metabolic Derangement

A primary deficiency of the lysosomal (or acid) sphingomyelinase (◘ Fig. 38.2) resulting from mutations in *SMPD1* leads to the progressive accumulation of sphingomyelin in systemic organs in all types of the disease, and in brain in the neuronopathic forms [34]. Sphingomyelin storage is massive in liver and spleen in type A, slightly less so in type B. A significant increase of unesterified cholesterol occurs secondarily [41]. By in vitro measurements a marked ASM deficiency is observed in all patients, but hydrolysis of sphingomyelin in live cells demonstrates a significant level of residual activity in typical type B patients, suggesting this could be enough to limit accumulation and protect the brain. Sphingosylphosphorylcholine

(lysosphingomyelin) (increased in systemic organs of all types and in type A brain) may participate in the pathogenic cascade.

Genetics

More than 180 disease-causing mutations of *SMPD1* have been reported [42]. In Ashkenazi Jewish type A patients, 3 mutations (R496L, L302P, P330fs) [now p.Arg498Leu, p.Leu304Pro, p.Phe333fs*52 (c.996delC)] account for >90% of alleles. R608del (now p.Arg610del), highly prevalent in North African patients, is the most common type B mutation (20–30% of alleles) in many countries. So far, it has always been correlated with a type B phenotype whatever the nature of the second mutated allele. Q292K (p.Gln294Lys) is associated with late-onset neurological involvement. *SMPD1* appears to be paternally imprinted.

Diagnostic Tests

Bone marrow usually reveals the presence of (nonspecific) foamy histiocytes or sea-blue histiocytes. Among plasma biomarkers, the oxysterols cholestane-3β,5α,6β-triol and 7-ketocholesterol, as well as lysosphingomyelin and ›lyso-sphingomyelin-509‹, are elevated [43][44]. Chitotriosidase is moderately elevated. Note that these biomarkers are also elevated in Niemann-Pick type C (▶ below) and for oxysterols, in acid lipase deficiencies and some other conditions [45][46]. The diagnosis is made by demonstration of a deficiency in ASM activity in leukocytes (or lymphocytes) or in cultured cells (much higher level of activity). The choice of a specific substrate is critical. Radioactively labelled native sphingomyelin or a short-chain analogue with tandem mass spectrometry measurement [47] are best. The fluorogenic substrate [48] should be used with caution [38]. The *in vitro* assay does not reliably distinguish A from B phenotypes.

Treatment and Prognosis

No specific therapy is yet available. Limited experience of bone marrow transplantation (BMT) in type A patients has not appeared to improve symptoms. In type B, splenectomy may have a deleterious effect on the lung disease. Pregnancy is not contraindicated in type B patients, although monitoring for bleeding is advisable. Morbidity/mortality of type B has been studied [49]. Encouraging results of an ERT phase 1 clinical trial in adult type B patients using olipudase alpha [50] have led to a phase 2 trial in children and adults.

38.2.3 GM1 Gangliosidosis

Clinical Presentation

First descriptions of infantile GM1 gangliosidosis emphasised its characteristics of a neurovisceral lipidosis sharing features with both Tay-Sachs disease and Hurler disease. Forms with an almost exclusive neuronal storage were described later. Three clinical phenotypes are recognised [51].

In the **typical early infantile form** (or type 1), children are often hypotonic in the first days or weeks of life, with poor head control. The arrest in neurological development is observed at 3–6 months of age. Feeding difficulties and failure to thrive are common. Many infants have facial and peripheral oedema. In typical cases, dysmorphic features may be present very early or develop with time, with a puffy face, moderate macroglossia, hypertrophic gums, depressed nasal bridge and chipmunk face, but an increasing number of infantile patients have presented without dysmorphic expression. Hepatomegaly and later splenomegaly are almost always present. Dorsolumbar kyphoscoliosis is common. After a few months, signs of visual failure appear, often with a pendular nystagmus. A macular cherry-red spot is found in about 50% of cases, but seldom before 6 months of age. As time passes, hypotonia gives way to spasticity. Rapid neurological regression is usual after the 1st year of life, with generalised seizures, swallowing disorder, decerebrate posturing and death, often before age 2. Radiological signs in the long bones and spine are constant in clinically severe patients, but can be minimal in cases with only psychomotor deterioration. Subperiosteal bone formation can be present at birth. Widening of the diaphyses and tapering of the extremities appear later. At the age of 6 months, striking Hurler-like bone changes are seen, with vertebral beaking in the thoracolumbar zone, broadening of the shafts of the long bones with distal tapering and widening of the metacarpal shafts with proximal pinching of the four lateral metacarpals.

A severe **neonatal form** with cardiomyopathy has been described. GM1 gangliosidosis is also a cause of nonimmune *foetal hydrops*.

The **late infantile variant** (or type 2) usually begins between 12 and 18 months (but up to 3 years), with unsteadiness in sitting or standing, or difficulty in walking. Regression is rapid and severe, and a spastic quadriparesis develops, associated with pseudobulbar signs. Seizures are frequent and may become a major problem. The patients are not dysmorphic, and hepatosplenomegaly is not present. Vision is generally normal. Radiography of the spine reveals moderate but constant changes, with mild anterosuperior hypoplasia of the vertebral bodies at the thoracolumbar junction.

The term ›**adult form**‹ has been employed to designate the **chronic late-onset form** of GM1 gangliosidosis with onset in late childhood, adolescence or adulthood. Dysarthria and extrapyramidal signs, especially dystonia, are the most common signs [52][53]. Cognitive impairment is absent to moderate, and there are no ocular abnormalities. Bone changes are inconstant. The course of the disease is very slow.

Metabolic Derangement

GM1 gangliosidosis is due to a deficient activity of lysosomal acid β-galactosidase (◘ Fig. 38.2), which cleaves glycoconjugates containing a terminal β-galactosidic linkage and is necessary for the degradation of GM1 ganglioside, other galactose-containing glycosphingolipids or oligosaccharides, as well as keratan sulfates (▶ Chapter 39). Consequently, the most severe forms of the disease combine features of a neuronal lipidosis, a mucopolysaccharidosis and an oligosaccharidosis. Acid β-galactosidase functions in a multienzyme lysosomal complex with neuraminidase, the protective protein/cathep-

sin A (PPCA) and N-acetyl-galactosamine-6-sulfate sulfatase [54]. This explains the quite similar clinical phenotype of galactosialidosis, a distinct condition due to the deficiency of PPCA, which causes a combined secondary deficiency of acid β-galactosidase and acid sialidase (neuraminidase) (▶ Chapter 39). Finally, β-galactosidase deficiency can be associated with two clinically different diseases, GM1 gangliosidosis, with prominent features of a sphingolipidosis, and Morquio B disease (mucopolysaccharidosis type IVB), in which abnormalities of mucopolysaccharide metabolism prevail. In tissues from patients with GM1 gangliosidosis, three main groups of accumulated compounds have been identified: the sphingolipid GM1 ganglioside, glycoprotein-derived oligosaccharides and keratan sulfate. Massive storage of GM1 occurs in brain tissue. Increased levels of its lysocompound, potentially of pathogenic significance, have been reported. Galactose-containing oligosaccharides have been found in liver and urine. Keratan sulfate and other mucopolysaccharides accumulate in liver and spleen. Keratan sulfate excretion in urine is lesser in GM1 gangliosidosis than in Morquio B disease.

▪ Genetics

About 150 mutations of *GLB1* (3p21.33) have been described. Neither the type nor location of the mutation correlate well with a specific phenotype.

▪ Diagnostic Tests

Vacuolated lymphocytes may be found in peripheral blood, and foamy histiocytes in the bone marrow. Radiographic bone examination showing Hurler-like abnormalities (▶ above) may suggest the diagnosis. In the infantile form, brain computerised tomography (CT) and magnetic resonance imaging (MRI) usually give nonspecific results, with diffuse atrophy of the central nervous system (CNS) and features of myelin loss in the cerebral white matter. Lesions in the basal ganglia may be present in the adult form. Analysis of urinary oligosaccharides is a good orientation test. In the classic early infantile form excretion is massive, with a pathognomonic profile. Excretion can be much lower in forms with predominant neurodegenerative disease. Mucopolysaccharide analysis in urine usually shows increased levels of keratan sulfate. The diagnosis is established by demonstration of a deficient activity of acid β-galactosidase, which can be measured on leukocytes or dried blood spots using an artificial fluorogenic substrate. A subsequent study of neuraminidase (in leukocytes or cultured fibroblasts) should be systematically performed to exclude galactosialidosis.

▪ Treatment and Prognosis

No specific treatment is available to date. SRT or chaperones are potential approaches for clinical trials in late-onset forms [51][55].

38.2.4 GM2 Gangliosidoses

GM2 gangliosidoses are divided into three genetic and biochemical subtypes: **Tay-Sachs disease** (or B variant), **Sandhoff disease** (or 0 variant), and **GM2 activator deficiency** (AB variant). All are characterised by impaired lysosomal catabolism of GM2 ganglioside (◘ Fig. 38.2), which requires three gene products: the β-hexosaminidase α- and β-subunits and the GM2 activator protein. Tay-Sachs disease corresponds to a deficiency of the α-subunit and thus of β-hexosaminidase A (αβ-heterodimer), Sandhoff disease, to a deficiency of the β-subunit and thus of both β-hexosaminidases A and B (ββ-homodimer). Classic Tay-Sachs disease has a very high carrier rate (estimated to ~1:27) in the Ashkenazi Jewish population, and also in subjects of French Canadian descent. Infantile forms are most common, but juvenile and adult forms are also recognised. A particular enzymatic variant of Tay-Sachs disease (B1 variant) has a high incidence in northern Portugal [56] and is globally more frequent in southern Europe. Variant AB is exceedingly rare (<10 reported cases), albeit probably underdiagnosed.

▪ Clinical Presentation

The **infantile** forms of the three subtypes have a very similar presentation [57]. Around 4–6 months of age, motor weakness and hypotonia are the usual earliest signs, almost constantly associated with a typical startle response to sounds with extension of the arms (hyperacusis). Hypotonia progresses, with loss of acquired milestones. Loss of visual attentiveness is also seen early, and ophthalmoscopic examination almost invariably reveals a typical macular cherry-red spot in the retina. Blindness follows, and spasticity, swallowing disorder and seizures develop. Macrocephaly begins by 18 months of age. By year 3 the child is demented and decerebrate. Death often occurs, due to aspiration pneumonia. In Sandhoff disease, in spite of an additional accumulation of glycolipids and oligosaccharides in visceral organs, organomegaly and bony abnormalities are rarely observed.

Late infantile and **juvenile** forms [57][58] are mostly due to a deficiency of β-hexosaminidase A (often B1 variant). The onset of symptoms is usually between 2 and 10 years of age, with ataxia, incoordination and dysarthria, followed by progressive psychomotor deterioration, spasticity and seizures. Myoclonus can be prominent. Cherry red-spots are inconstant.

Chronic or **adult** forms can show variable presentations, with pyramidal and extrapyramidal signs, movement disorders (dystonia, athetosis, ataxia), psychosis (reported in 30–50% of adult-onset patients) and a syndrome of lower motor neuron and spinocerebellar dysfunction with supranuclear ophthalmoplegia [59][60]. Some patients show autonomic dysfunction.

▪ Metabolic Derangement

The normal catabolism of GM2 ganglioside requires the GM2 activator protein to extract GM2 from the plasma membrane before presenting it to hexosaminidase A (αβ-heterodimer). Hexosaminidase B (ββ-homodimer) hydrolyses other sub-

strates with a terminal hexosamine (glycoproteins and glycolipids), but not GM2 ganglioside. In Tay-Sachs disease (affecting the α-subunit), hexosaminidase A only is deficient. In Sandhoff disease (affecting the β-subunit) both hexosaminidases are inactive. In GM2 activator deficiency, the substrate is not made available to the otherwise normally functioning enzyme. All types are characterised by storage of GM2 ganglioside in neurons. This results in meganeurites, with aberrant neurite formation that may play a role in the pathophysiological mechanisms. GM2 storage is very pronounced in infantile forms, less so in juvenile forms, and even less in adult forms. Increased levels of lyso-GM2 have also been reported in infantile forms. In Sandhoff disease, asialo-GM2 also accumulates in brain, while other compounds – such as globoside and oligosaccharides – accumulate in liver and other visceral organs.

Genetics

More than 130 mutations of *HEXA* have been identified. Three of them – c.1274-1277dup, c.1421+1G>C in infantile cases, p.Gly269Ser in adult forms – account for>95% of the Ashkenazi Jewish alleles. A carrier screening programme initiated in the early 1970s has proven very successful to decrease incidence of the disease in this population. A 7.6 kb deletion is common in French Canadian patients. Mutations at codon 178 altering the three-dimensional structure of the enzyme, result in the enzymatic B1 variant presenting as a juvenile form in the homozygous state. Relatively good genotype-phenotype correlations have been reported. More than 40 mutations (including a common 16 kb deletion) in *HEXB* and 6 in the GM2 activator *GM2A* gene have been described.

Diagnostic Tests

In Tay-Sachs and Sandhoff diseases, the clinical diagnosis can easily be confirmed by enzyme testing on leukocytes or cultured fibroblasts. The assay for total hexosaminidases (A+B) using a synthetic fluorogenic substrate is straightforward and allows the diagnosis of Sandhoff disease. Differential assay of hexosaminidase A using heat or acid inactivation does not identify patients with the B1 variant; the direct assay of hexosaminidase A using the sulfated synthetic substrate (4-MU-6-sulfo-β-glucosaminide) specific for the α-subunit is the method of choice. A high residual activity is found in Sandhoff disease, owing to excess of hexosaminidase S (αα-dimer). In GM2 activator deficiency, hexosaminidase A activity measured in vitro is normal; electron microscopic examination of a skin or conjunctival biopsy may provide strong evidence in favour of the diagnosis by demonstrating concentric lamellated bodies in nerve endings. The CSF shows increased levels of GM2. The definitive diagnosis requires *GM2A* sequencing.

Treatment and Prognosis

Seizures are generally responsive to standard treatment. No effective curative treatment is currently available. Neither SRT (miglustat) [61][62], nor chaperone therapy (pyrimethamine) [63] trials led to measurable clinical improvement. A gene therapy clinical trial is underway in the United Kingdom and the United States.

38.2.5 Krabbe Disease

Clinical Presentation

Krabbe disease (or globoid cell leukodystrophy) leads to demyelination of the central and peripheral nervous system. Its estimated overall incidence is between 0.75 and 1 in 100,000 live births. It is more frequent in Scandinavia (but not in Finland). The classic early infantile form accounts for about 65% of diagnosed cases. Late onset cases appear to be more common in southern Europe, especially Italy and Sicily, and in Japan. The incidence of adult-onset cases has been underestimated.

Infantile forms Clinical presentation is quite uniform, usually very suggestive of the diagnosis.

In the **early infantile** form [64], the onset is from birth to 6 months of age (often 3–4 months) [65]. Initial symptoms include increasing irritability, crying, vomiting and other feeding problems, hyperesthesia, tonic spasms on light or noise stimulation, and signs of peripheral neuropathy. Bouts of unexplained fever are also common. This stage with hypertonic episodes is followed by permanent opisthotonic posturing with characteristic flexed upper extremities and extended lower extremities. Seizures may appear. Hyperpyrexia and hypersalivation are frequent. As the disease progresses blindness occurs, followed by loss of bulbar functions and hypotonia. Death occurs from hyperpyrexia, respiratory complications or aspiration, classically before the age of 2 years but in current practice not so rarely later.

In the **late infantile** phenotype (onset between 7–12 months, about 10% of cases), patients typically present with a loss of developmental milestones and poor feeding, crying and irritability being later signs [66].

Later onset forms Clinical recognition of these forms is more difficult.

The **juvenile** form [66] starts between the ages of 13 months and 10 years (in most cases before the age of 5 years). The first signs are often gait disturbances (spastic paraparesis or ataxia or both, sometimes spastic hemiplegia) in a previously normal or mildly retarded child. Visual failure with optic atrophy is also a common symptom in younger children [66][67]. At variance with the infantile form, peripheral neuropathy is only present in approximately half of the cases. Time of onset and severity of mental deterioration are variable. Seizures are infrequent; when present they can be a major therapeutic problem. The course of the disease is quite variable and unpredictable, even in siblings. Many patients show initial rapid deterioration followed by gradual progression lasting for years.

Most **adult** patients (reviewed in [68]) present with a gait disorder, showing a pyramidal syndrome with spastic paraparesis, with or without peripheral neuropathy. One third have cerebellar ataxia in addition. Usually they do not

show cognitive dysfunction. At MRI, hyperintensities along the pyramidal tracts are a characteristic and nearly constant sign.

Metabolic Derangement

Krabbe disease (Fig. 38.2) results from β-galactosylceramidase (or galactocerebrosidase, cerebroside β-galactosidase) deficiency, a lysosomal enzyme that catabolises (β-)galactosylceramide – a major lipid component of myelin – as well as lactosylceramide and galactosylsphingosine (psychosine). In vivo, galactosylceramide degradation further requires the saposin sap-A. Two cases due to sap-A deficiency are known [69]. Galactosylceramidase deficiency leads to an accumulation of galactosylceramide in the pathognomonic ›globoid cells‹ (multinucleated macrophages) seen in the demyelinating lesions of the white matter, and of a toxic metabolite, galactosylsphingosine (psychosine) in the oligodendrocytes and the Schwann cells. Psychosine, a highly apoptotic compound increased in the brain of infantile patients, is thought to play a major role in the pathogenesis of the disease and, more specifically, to underlie the early destruction of oligodendrocytes characteristic of the infantile form, and thus an arrest of myelin formation [70].

Genetics

More than 150 *GALC* mutations are known. The most frequent mutant allele (never found in Japanese patients) combines a large (30-kb) deletion and the polymorphism 502C>T (now c.550C>T); G270D (now p.Gly286Asp) is frequent among adult-onset patients [68][71][72]. Some common polymorphisms – in particular 1637T>C (now c.1685T>C) and 502C>T – influence enzyme activity and may be responsible for a pseudodeficiency state, particularly when in compound heterozygosity with a disease-causing allele [71]. Two unrelated infantile cases were assigned to a mutation in the sap-A domain of *PSAP*.

Diagnostic Tests

Motor nerve conduction velocities are consistently low in infantile and most late infantile cases, but only about 60% of juvenile or adult patients display signs of peripheral neuropathy. MRI shows areas of hyperintensity on T_2-weighted images that correlate well with areas of demyelination and globoid cell accumulation [73]. In late-onset cases, T_2-weighted images may show more localised areas of hyperintensity with less involvement of cerebellum and deep grey matter [74][75]. In adult-onset cases, typical T_2 hyperintensities along the pyramidal tracts involving optic radiations and corticospinal tracts are nearly constant [68]. In typical infantile cases, CT shows diffuse cerebral atrophy with hypodensity of the white matter. Calcifications may be observed in the thalamus, basal ganglia and periventricular white matter. Brain stem evoked potentials have also been studied [76]. Protein in CSF is usually elevated in infantile cases, but inconstantly in late-onset cases. The ultimate diagnosis is made by studying galactosylceramidase activity in leukocytes, dried blood spots or cultured fibroblasts. This assay is subject to pitfalls of either technical (substrate) or biological (pseudodeficiency) nature. Use of a natural radiolabelled substrate is the gold standard, and a short-chain analogue (final measurement by tandem mass spectrometry) has also shown good specificity [47][77]. Published experience remains limited regarding a less sensitive fluorogenic substrate. In Krabbe disease, like in metachromatic leukodystrophy (▶ below), a pseudodeficiency state is relatively common and can lead to misinterpretation of correct data. For this reason, study of both parents is particularly important. Genotyping of all patients is recommended as prenatal diagnosis using molecular genetics is today preferred to enzymatic studies. In the two known patients with sap-A deficiency, galactosylceramidase activity was deficient in leukocytes but not in cultured fibroblasts (sap-A may stabilise galactosylceramidase).

Treatment and Prognosis

In advanced disease, supportive analgesic treatment of the often severe pain that can result from radiculopathy is important, as is treatment of spasticity. Allogenic BMT or cord blood transplantation may be effective in preventing onset or halting progression of the disease in late-onset cases [78]. In symptomatic infantile cases BMT gives poor results, unless performed presymptomatically. Initial results with umbilical cord blood transplantation to 12- to 44-day-old babies were very promising [79], leading to newborn screening in 2 states in the USA [77]. However, long-term follow-up indicated that over time most children developed slowly progressive motor and language deterioration along with somatic growth failure and persistent cognitive deficits [80][81]. Diffusion tensor imaging tractography might help in predicting neurodevelopmental outcomes in neonates with the disease [82].

38.2.6 Metachromatic Leukodystrophy

Clinical Presentation

Metachromatic leukodystrophy (MLD) is panethnic, with reported incidences ranging between 1 in 40,000 and 1 in 170,000, except in specific ethnic groups with higher frequency.

The **late infantile** form [83][84] is the most common. First symptoms appear between the ages of 1 and 2 years (median onset 18 months): walking delay, progressive difficulty in locomotion around 14–16 months (weaker lower limbs and falls); 15% of children never walk independently. Examination usually shows hypotonia, reduced or absent deep tendon reflexes and extensor plantar responses. Walking and then standing soon become impossible. The child develops spastic quadriplegia, speech deterioration, gradual mental regression and optic atrophy leading to blindness, followed by a vegetative state and death.

The age at onset of the **juvenile** form [84] ranges between 2.5 and 14 years. Some authors differentiate early (onset between 2.5 and 3.5 years) and late juvenile forms. Failure in school, behavioural problems or disturbance of cognitive function may precede motor abnormalities, especially in patients with a later onset (>6 years). Progressive difficulties in walking,

with pyramidal signs and peripheral neuropathy, together with cerebellar ataxia constitute the most common presentation, but various other symptoms can occur, such as hemiplegia, dystonia and choreoathetosis. Seizures may also develop.

A severity scoring based on a gross motor function classification has been developed for late infantile and juvenile forms. Age of entry into the different stages and dynamics of decline of gross motor function have been reported [85], as well as natural course of language and cognition [86].

Two distinct types of **adult** MLD have been identified [87]. In the first group, patients have predominant motor disease, with pyramidal and cerebellar signs, dystonia and peripheral neuropathy, or isolated peripheral neuropathy. In the second group, behavioural and psychiatric problems (often confused with schizophrenia) are the presenting symptoms, followed by dementia and spastic paresis [88].

Metabolic Derangement

The primary metabolic defect is a block in lysosomal degradation of sulfatide (or galactosylceramide-sulfate) and other sulfated glycolipids (Fig. 38.2). *In vivo*, the sulfatide is presented to the enzyme arylsulfatase A (ASA) as a 1:1 complex with sap-B. A deficiency of either ASA or sap-B can cause MLD. Few cases with sap-B deficiency have been documented, most with a late infantile form. Sulfatide is a prominent lipid component of the myelin sheath. Its ratio to galactocerebroside plays a role in the stability and physiological properties of this membrane. Progressive accumulation of sulfatides (and likely lysosulfatide) in the central and peripheral nervous system will soon lead to disruption of the newly formed myelin and intense demyelination. In MLD, sulfatide also accumulates in the kidney, which is reflected in a highly abnormal excretion of sulfatide in urine sediment.

Genetics

About 200 different *ARSA* mutations are known [89]. The three more frequent alleles among European patients are c.465+1G>A (traditional denomination 459+1G>A) (severe phenotype), p.Pro428Leu (P426L) (mild phenotype) and p.Ile181Ser (I179S) (mild phenotype). There is a relatively good genotype-phenotype correlation [84]. Two very frequent *ARSA* polymorphisms, one leading to the loss of an *N*-glycosylation site and the second to the loss of a polyadenylation signal, result in reduction of the amount of enzyme and constitute the molecular basis of ASA pseudodeficiency [89]. They often occur jointly, but can also be found independently. In some countries, as many as 15% of the general population carry one allele with such a pseudodeficiency (*pd*) [84]. MLD due to sap-B deficiency is panethnic, but seems more frequent in Saudi Arabia, Turkey and North Africa. These patients have mutations (10 described to-date) in *PSAP*.

Diagnostic Tests

In most patients, motor nerve conduction velocities of peripheral nerves are decreased and sensory nerve action potentials have a diminished amplitude with a prolonged peak latency [90]. Decreased nerve conduction is not always present in adult MLD. MRI shows similar fairly characteristic symmetrical changes of the central white matter in all forms. A sheet-like area of abnormal T_2 signal hyperintensity initially envelops the frontal and parietal periventricular and central white matter regions, faint in mild disease and denser in moderate to severe disease. As severe disease develops, the sheet of white matter signal intensity abnormality also involves the inner half of the subcortical white matter, and a tigroid pattern emerges [91][92]. The late infantile form also involves cerebral atrophy. Abnormalities are also described by diffusion MRI and proton magnetic resonance spectroscopy (MRS). The CSF protein content is usually elevated in late infantile patients (although not at an early stage), inconstantly in the juvenile form and rarely in the adult form.

Determination of ASA activity in leukocytes (or cultured fibroblasts) using *p*-nitrocatechol-sulfate as a substrate constitutes the first biochemical test. Pseudodeficiency is a major pitfall [84]. Individuals homozygous for a *pd* allele (1–2% of the European population) or subjects compound heterozygotes for a disease-causing *mld* and a *pd* allele have about 5–15% of normal ASA activity but no detectable clinical abnormality or pathology. Deficient ASA activity is therefore not enough to conclude to the diagnosis of MLD. The study of sulfatides in the urinary sediment circumvents the problem. MLD (but also multiple sulfatase deficiency, see ▶ below) patients excrete massive (late infantile and juvenile patients) or significant (adult-onset type) amounts of sulfatides, while subjects with an ASA pseudodeficiency have levels within or slightly above the normal range. ASA pseudodeficiency also poses problems in genetic counselling. In a newly diagnosed family, it is important to measure enzyme activity in both parents. Full genotyping of the index case and study of parental DNA are highly recommended. Prenatal testing of MLD by DNA analysis is now the preferred strategy in many laboratories.

Another cause of erroneous interpretation of an ASA deficiency is **multiple sulfatase deficiency** (MSD), due to a deficiency in the formylglycine-generating enzyme (FGE) encoded by *SUMF1*. Whenever a deficiency of one sulfatase is found, it is mandatory to systematically measure the activity of another one (here, arylsulfatase B or iduronate-2-sulfatase) to exclude MSD, as the clinical picture can be misleading.

In MLD patients with sap-B deficiency, the in vitro ASA assay will not show a deficiency. Studies of sulfatides and globotriaosylceramide (Gb3) excretion in urine are essential. Both lipids are elevated (combined MLD and Fabry pattern). The definitive diagnosis will require *PSAP* molecular genetics study.

Treatment and Prognosis

Symptomatic treatment of spasticity and of pain resulting from radiculopathy is important. Allogenic HSCT has been performed in a number of cases. It is generally considered that adult-onset and juvenile-onset patients benefit, with slowing of the disease progression and improvement of cognitive functions, but challenging reports have appeared [84][93][94][95][96]. Whether HSCT is indicated in the late infantile form remains controversial [84]. Symptomatic patients are not can-

didates; a few presymptomatic affected siblings have received HSCT, with significant difference in survival and CNS involvement compared with untransplanted siblings, but no effect on the peripheral neuropathy. More recently, three presymptomatic patients received lentiviral hematopoietic stem cell gene therapy with good results at evaluations up to 18 or 24 months post-treatment [97]. In late infantile patients, a phase 1/2 clinical trial with intrathecal administration of rhASA is ongoing, as well as a phase 1/2 trial with intracranial administration of AAV10-rhASA.

38.2.7 Fabry Disease

Clinical Presentation

Fabry disease, the only X-linked sphingolipidosis, is associated with severe multiorgan dysfunction [98][99][100]. Its incidence is estimated at 1:40,000 to 1:60,000 live births for males, but from neonatal screening studies [101], late onset phenotypes appear largely undiagnosed. Of note, many heterozygous females are symptomatic. Males with the classic form have a disease onset during the 1st decade, typically with crises of severe pain in the extremities (acroparesthesia) provoked by exertion or temperature changes, that may last hours to days. Unexplained bouts of fever and hypohidrosis, heat, cold and exercise intolerance, gastrointestinal problems and corneal dystrophy (cornea verticillata) not affecting vision, are other manifestations. At this stage, renal function, urinary protein excretion and cardiac function and structure are generally still normal [102]. Characteristic skin lesions, angiokeratomas, appear on the lower part of the abdomen, buttocks and scrotum in 80% of patients. Progressive renal involvement, which may result in end-stage renal disease and require dialysis or transplantation, occurs in adulthood. Cardiac manifestations include left ventricular hypertrophy, valvular disease (mitral insufficiency), ascending aortic dilatation, coronary artery disease and conduction abnormalities leading to congestive heart failure, arrhythmias and myocardial infarction. Cerebrovascular manifestations include early stroke, transient ischaemic attacks, white matter lesions, hemiparesis, vertigo or dizziness, and complications of vascular disease, in particular hearing loss. A recent survey indicates that symptoms such as acroparesthesias, neuropathic pain, gastrointestinal problems can occur even in early childhood (before 5 years of age) [103]. Clinical manifestations in heterozygous females range from asymptomatic to full-blown disease, as severe as in affected males but with globally a later onset and slower progression. A growing number of patients with atypical cardiac, renal or cerebrovascular disease variants with a milder, later onset phenotype or single organ involvement have been described (for a comprehensive review see [98]). Screenings have been conducted in »high-risk« populations [104].

Metabolic Derangement

The primary defect is a deficient activity of the lysosomal enzyme α-galactosidase A, which releases galactose from ceramide trihexoside (globotriaosylceramide, Gb3) and elevated glycosphingolipids (especially galabiosylceramide, Gb2), due to mutations of the *GLA* gene (◘ Fig. 38.2). This results in progressive accumulation of Gb3 in vascular endothelial cells, perithelial and smooth muscle cells, leading to ischaemia and infarction especially in the kidney, heart and brain. Early and substantial deposition of Gb3 occurs in podocytes, leading to proteinuria, and with age, in cardiomyocytes, causing cardiac hypertrophy and conduction abnormalities. Small-fibre polyneuropathy is the cause of pain and anhidrosis. Lysosomal storage and cellular dysfunction are believed to trigger a cascade of events resulting in tissue ischaemia and development of irreversible cardiac and renal tissue fibrosis [98].

Genetics

Fabry disease has an X-linked recessive transmission. Adequate genetic counselling in the family, including female carrier detection, is therefore essential. Nearly 1000 variations of *GLA* are known, and defining their pathogenicity remains a crucial problem, especially in screening programmes [105]. Many mutations are private; a number are recurrent in specific countries. The p.Asn215Ser mutation seems to be associated with the cardiac variant. De novo mutations are rare. In females, the X-chromosome inactivation pattern seems more contributive to disease expression than the mutation itself [106].

Diagnostic Tests

In affected males with the classic or variant phenotype, the disease is readily diagnosed by showing a deficient α-galactosidase A activity in leukocytes. Dried blood spots are better suited to large-scale screening, but subsequent confirmation in leukocytes is essential. Heterozygous females show normal to low levels of activity; enzyme assay is thus not reliable for carrier detection, and studying the family mutation is the test of choice in subjects related to a patient with definite diagnosis. Results of *GLA* sequencing alone can often be difficult to interprete in cases of suspected Fabry disease [107]. In urinary sediment, Gb3 and Gb2 are excreted in large amounts by untreated male hemizygotes (except those with a renal graft or with a cardiac variant), and in smaller amounts by 90% of heterozygous females, symptomatic or not. Plasmatic lyso-Gb3 [105][108] is recognized as a sensitive and useful biomarker. Definite diagnosis of Fabry disease should combine several biological and clinical criteria; in atypical – particularly cardiac – variants, electron microscopic study of the target organ may be necessary [107].

Treatment and Prognosis

The disease results in a significant reduction in life expectancy due to renal disease and cardiovascular or cerebrovascular complications [98]. There is also the psychosocial burden of a rare, chronic and progressive disease. Alleviation of pain and treatment of the renal and cardiac disease are important issues. Dialysis or renal transplantation may be necessary for patients with end-stage renal failure. There is now a long-term experience of ERT with recombinant α-galactosidase A products (agalsidase alpha or agalsidase beta). The oral pharmacological chaperone migalastat has recently been approved in the

EU for treatment of patients aged 16 years or older with an amenable mutation. ERT leads to improvement for many of the symptoms and appears efficient in reducing left ventricular hypertrophy, with lesser effect on renal function. But in long-term studies, it did not prevent progression [109][110][111]. A European consortium has proposed recommendations for initiation and cessation of ERT [111].

38.2.8 Farber Disease / Acid Ceramidase Deficiency

Clinical Presentation

The very rare »Farber lipogranulomatosis« is clinically heterogeneous. It often presents during infancy causing death within the 1st year, but later onset cases (up to an adult age) have been described, as well as foetal forms [112]. The most frequent signs are painful joint swelling, deformation and contractures, periarticular subcutaneous nodules and hoarseness due to laryngeal involvement. The presentation of some patients mimics juvenile idiopathic arthritis [113]. Hepatomegaly and a macular cherry-red spot may be present. Neurological manifestations are of variable severity (from mild to psychomotor deterioration and epilepsy); juvenile-onset patients may show neurological involvement only. A distinct form of acid ceramidase deficiency showing spinal muscular atrophy and progressive myoclonic epilepsy (SMA-PME) has been delineated [114], with more cases being recently described [115].

Metabolic Derangement and Genetics

The deficiency of acid ceramidase activity leads to the storage of ceramides in various organs [116]. More than 30 mutations of *ASAH1* have already been described [117], including a large deletion.

Diagnostic Tests

Electron microscopy of an excised nodule or of a skin biopsy may reveal inclusions with typical curvilinear bodies in histiocytes, and ›banana bodies‹ in Schwann cells. In vitro measurement of ceramidase activity requires a specific substrate available in only few laboratories [118]; so are ceramide precursors loading tests in living fibroblasts or ceramide levels determinations. It is therefore often easier and quicker to directly sequence *ASAH1*.

Treatment and Prognosis

Currently there is no specific therapy. Good results of BMT have been reported only in patients without CNS involvement [119]. Development of ERT and gene therapy is being facilitated by the recent availability of a suitable mouse model.

38.2.9 Prosaposin Deficiency

Clinical Presentation

The eight published cases have shown almost the same course, with severe neurovisceral storage disease manifesting immediately after birth with rapidly fatal course and death between 4 and 17 weeks of age. The patients have hepatosplenomegaly, hypotonia, massive myoclonic bursts, abnormal ocular movements, dystonia and seizures [120].

Metabolic Derangement and Genetics

Sphingolipid activator proteins are small glycoproteins that are required as cofactors for the lysosomal degradation of sphingoglycolipids with short hydrophilic head groups and ceramide. They act either by solubilising the substrate or by mediating enzyme binding to the membrane or modifying the enzyme conformation. *PSAP* encodes the prosaposin protein, which is transported to the lysosome where it is processed to four homologous proteins. Sap-A is a cofactor for degradation of galactosyl- and lactosylceramide; its deficiency causes a Krabbe disease variant (2 cases known); sap-B is involved in the in vivo degradation of sulfatides and Gb3, and its deficiency causes an MLD variant (>25 cases known); sap-C is necessary for hydrolysis of glucosylceramide, and its deficiency causes a Gaucher disease variant (5 cases known). Although no patient has been described with sap-D deficiency, this factor is implicated in ceramide degradation. Prosaposin deficiency is due to the combined lack of all four sap- factors, explaining tissue storage of all the lipids cited above. The disorder is autosomal recessive. Mutations identified in patients explain abolished production of the prosaposin precursor and thus of all four factors.

Diagnostic Tests

Gaucher-like cells are found in bone marrow. Study of glycolipids in urine sediment shows a pattern close to that described for sap-B deficiency. Galactocerebrosidase activity has been reported to be deficient in leukocytes and fibroblasts. Lipid studies in liver tissue revealed a combined increase of glucosylceramide, lactosylceramide and ceramide. The loading test in living fibroblasts described for Farber disease shows a severe block in ceramide hydrolysis. In practice, the abnormal typical profile of urinary glycolipids and/or elevated plasma lyso-Gb3 and lyso-glucosylceramide should lead to complete *PSAP* sequencing.

38.3 Niemann-Pick Disease Type C

38.3.1 Clinical Presentation

Niemann-Pick disease type C (NP-C) is panethnic, with an estimated incidence around 1 in 100,000 [121][122]. The clinical course is extremely heterogeneous and age at presentation varies from the perinatal period to late adulthood. Visceral involvement (liver, spleen and lung) and neurological or psychiatric manifestations arise at different times, and they follow an independent course. Systemic disease, when present, always precedes the onset of neurological symptoms; the systemic component may decrease with time, be minimal, or absent. Apart from a small subset of patients who die in the perinatal period and exceptional adult cases, all patients

ultimately develop a progressive and fatal neurological disease. For periods other than perinatal, some patients show only systemic signs, while others start to show neurological symptoms. A classification by neurological form (rather than by age at disease onset) is widely used, because a correlation between age at neurological onset and following course of disease and life-span has been established [122].

▪ Perinatal Presentations

Foetal period Foetal hydrops or foetal ascites (often with splenomegaly) can occur.

Neonatal period In early life, liver involvement is often present. About one third of NP-C patients show a *prolonged neonatal cholestatic icterus* with hepatosplenomegaly. In most patients, the icterus resolves spontaneously and only hepatosplenomegaly remains [122]. Such patients may later develop any neurological form, although rarely an adult onset form [123]. In a few infants, the liver disease worsens and they die from hepatic failure before 6 months of age, defining a **neonatal, cholestatic rapidly fatal form**. Isolated *hepatosplenomegaly or splenomegaly* can also start at this period. A few infants develop a severe respiratory insufficiency [122].

▪ Period with Isolated Systemic Symptoms

Isolated splenomegaly or hepatosplenomegaly can be the first sign of disease, and be detected at any age. Onset of neurological symptoms may be protracted by many years. NP-C at this stage is one of the critical differential diagnoses of Niemann-Pick type B. A handful of adults up to 60 years of age have been described with systemic disease only [122]. More such cases may stay undiagnosed [121].

▪ Neurological Forms

Early infantile form In the severe early infantile neurological onset form, infants with a pre-existing hepatosplenomegaly (often with a history of neonatal cholestatic jaundice) show an early delay in motor milestones that becomes evident between the ages of 9 months and 2 years, and hypotonia. Most never learn to walk. The mental status is less severely affected. A loss of acquired motor skills is followed by spasticity with pyramidal tract involvement and mental regression. Signs of white matter involvement are present. Survival rarely exceeds 6 years [122].

Late-infantile- and juvenile-onset neurological forms (classic NP-C, 60–70% of cases) In the late infantile form, hepatosplenomegaly has generally been present for a varying period, but may be absent. Language delay is frequent. At the age of 3 to 5 years, the first obvious neurological signs are gait problems and clumsiness, due to ataxia. The motor problems worsen, cognitive dysfunction appears. In the juvenile form, onset of neurological disease is between 5–6 and 12 years, with more insidious and variable symptoms. Splenomegaly is variable. School problems, with difficulty in writing and impaired attention, are common and may lead to misdiagnosis. The child becomes clumsier with increasing learning disabilities, and obvious ataxia. In both forms, vertical supranuclear gaze palsy, with an increased latency of initiation of vertical saccades, is constant when correctly assessed and a characteristic sign. Gelastic cataplexy occurs in about 20% of patients and can be the presenting symptom. As ataxia progresses, dysphagia, dysarthria and dementia develop. Action dystonia is also frequent. About half of the patients develop seizures, which may become difficult to treat. In a later stage, the patients develop pyramidal signs and spasticity and severe swallowing problems. Most require gastrostomy. Death usually occurs between 7 and 12–14 years of age in late-infantile-onset patients, and is very variable in the juvenile form, some patients being still alive by age 30 or more [122][123].

Adolescent/adult onset form Age at diagnosis varies between 15 and 60 years or more. In adult-onset patients, presentation is even more insidious and diagnosis seldom made at an early stage. Atypical signs may in retrospect have been present since adolescence. Major signs are ataxia, dystonia and dysarthria, movement disorders, with variable cognitive dysfunction; psychiatric symptoms and dementia are dominant in certain patients [123][124]. In recent cohorts, vertical gaze palsy was a nearly constant sign. Epilepsy is rare in adult NP-C. Splenomegaly is inconstant.

38.3.2 Metabolic Derangement

NPC2 is a small soluble lysosomal protein which binds cholesterol with a high affinity; NPC1 is a large transmembrane protein with a main late endosomal localisation. When either protein is non functional, the cellular trafficking of endocytosed LDL-derived cholesterol is impaired, resulting in accumulation of unesterified cholesterol in the endosomal/lysosomal system and delay in homeostatic reactions. This specific abnormality constitutes the basis for biological diagnosis of NP-C. The two proteins act in sequence. In the prevailing »hand-off« model, cholesterol first binds to NPC2. NPC2 transfers it to NPC1, which facilitates its lysosomal egress by a still unknown mechanism [41][122][125]. However, the complete functions of NPC2, and above all of NPC1, remain unclear. In extraneural organs, the lipid storage pattern includes, besides unesterified cholesterol, sphingomyelin, several glycolipids, sphingosine and bis(monoacylglycero)phosphate, with no prevailing compound. Sphingolipid accumulation could be secondary to cholesterol storage [41]. In brain, neurons store significant amounts of glycolipids, particularly GM2 and GM3 gangliosides; but, despite clearly abnormal filipin staining in neurons, there is no quantitative increase of cholesterol (nor of sphingomyelin) in grey matter [41][125]. Main pathologic changes in brain, besides neuronal storage, are a prominent loss of Purkinje cells, neuroaxonal dystrophy, neurofibrillary tangles, meganeurite formation and ectopic dendritogenesis. Signs of delay in myelination and severe myelin loss are only prominent in the early infantile neurological form. The role of the more recently described block in Ca^{2+} release from acidic compartments in the pathogenic cascade is not well understood [41][122][125].

38.3.3 Genetics

Approximately 95% of patients harbour mutations in *NPC1*, the remainder in *NPC2*. More than 350 disease-causing *NPC1* mutations are already known, as are >100 polymorphisms. The most frequent mutant allele in patients of western European descent is p.Ile1061Thr, followed by p.Pro1007Ala. Some 50 families are known with *NPC2* mutations. Studies in multiplex families indicate that mutations correlate with the global neurological form rather than with the systemic manifestations. Certain mutations (e.g. p.Pro1007Ala) are associated with a lesser block in cholesterol trafficking (›variant‹ filipin test, see diagnostic tests section) [122][126].

38.3.4 Diagnostic Tests

Neuroimaging is generally not contributive to the diagnosis. Foamy and sea-blue histiocytes may (not always) be found in bone marrow aspirates. In plasma, the oxysterols cholestane-3β,5α,6β-triol and 7-ketocholesterol as well as ›lysosphingomyelin-509‹ are elevated in most cases [43][127] (as in ASM deficiencies), while lysosphingomyelin (also high in ASM deficiencies) shows a modest or no elevation [128]. The bile acid derivative 3β,5α,6β-trihydroxy-cholanoyl-glycine is also increased in NPC and ASM deficiencies. Note that oxysterols are also elevated in acid lipase deficiencies and some other diseases including neonatal cholestasis [45][46]. The definitive biochemical diagnosis requires live cultured fibroblasts. After culture in an LDL-enriched medium, pathognomonic accumulation of free cholesterol in lysosomes can be visualised by fluorescence microscopy after staining with filipin [122]. This »filipin test« will give unequivocal results in about 85% of patients, while interpretation is more difficult in the remainder, described as ›variants‹. In the latter, cholesterol accumulation is less prominent and not present in all cells, and complementary gene sequencing may be necessary to conclude. Today, positive biomarker testing is usually directly followed by molecular genetic analysis. The filipin study can be helpful to define the pathogenic nature of new gene variations or if mutations remain unidentified. Characterization of mutations may require more than DNA sequencing (large deletions, deep intronic mutations). Genotyping all patients is essential, as only the molecular genetics approach is now used for prenatal diagnosis [122][129]. For review see [130].

38.3.5 Treatment and Prognosis

Cataplectic attacks can be treated by clomipramine or CNS stimulants. Management of epilepsy, when present, is essential. With progression, most patients will require tube feeding or gastrostomy [129][131]. To date, miglustat is the only treatment specifically approved for neurological manifestations of NP-C, in the EU and many other countries (but not in the USA). Indications, clinical utility and monitoring have been discussed [129][131]. Initial data indicating stabilisation of patients for one year or more [132][133] and a slower rate of progression of the disease after treatment [134] have been confirmed; patients with later onset forms appear as better responders [135]. There is a rationale for HSCT in NP-C2 patients (at variance with NP-C1), but (early) follow-up is known in only one patient. Following encouraging preclinical studies in the *Npc1* feline model [136], a phase 1/2 trial with intrathecal administration of 2-hydroxypropyl-β-cyclodextrin is being completed; a phase 2b/3 is starting. Another clinical trial with oral administration of arimoclomol, a heat shock protein (hsp70 and hsp40) enhancer, is also underway.

38.4 Neuronal Ceroid Lipofuscinoses

Neuronal ceroid lipofuscinoses (NCLs) are a group of inherited progressive neurodegenerative diseases, among the most frequent in childhood. The term NCL is widely used in Europe, but the generic term »Batten disease« is common in the USA. The first description dates back to 1826, and since then, many clinical forms have been reported in the literature, strongly suggesting a large heterogeneity of the disease. The past 20 years have seen major advances in the field and the clinical diversity has now been linked to a wide genetic heterogeneity, with already 13 different genes identified, and probably more to come. Five of them encode soluble proteins, the others encode transmembrane proteins whose function and possible interactions still remain incompletely understood. NCLs are now considered as lysosomal storage diseases, due to the lysosomal accumulation of lipopigments, and above all, the more recent localisation of several NCL proteins to the lysosome.

38.4.1 Clinical Presentation

NCLs are usually characterised by progressive psychomotor retardation, seizures, visual loss and early death. Four main clinical forms have been described according to the age of onset and the order of appearance of clinical signs : infantile, late infantile (the most common in South Europe), juvenile (common in Anglo-Saxon countries) and adult (rare) [137]. However, numerous other clinical variants have been reported. This clinical heterogeneity is related to the diversity of the genes involved and to the variable severity of mutations. Therefore, the first classification based on the clinical forms has now been replaced by a new one using the genetic loci and including various forms with different ages of onset even if one form is usually predominant for each gene [138] (◘ Table 38.2).

- **Classic Infantile Neuronal Ceroid Lipofuscinosis (INCL, Santavuori-Haltia) Linked to *PPT1* (*CLN1*)**

Its incidence is high in Finland (1 in 20,000). Children with INCL are normal at birth. Symptoms usually begin between 6 and 24 months. They include delayed development, hypotonia, deceleration of head growth, seizures and jerks. Sleep disturbances are seen in most children. Rapid visual impairment occurs due to optic atrophy and macular degeneration. Stereo-

Table 38.2 Classification of NCLs with the corresponding genes, proteins and clinical forms. The different loci are organized by age of onset of the main clinical form (indicated in bold). The non-NCL phenotypes are given in italics

Gene	Protein	Clinical forms
CTSD (or ***CLN10***)	Cathepsin D	**Congenital** Late infantile, juvenile, adult
PPT1 (or ***CLN1***)	Palmitoyl protein thioesterase 1 (PPT1)	**Classic infantile** Late infantile, juvenile, adult
KCTD7 (or ***CLN14***)	Potassium channel tetramerization domain-containing protein 7 (KCTD7)	**Infantile** *Progressive myoclonic epilepsy* *Opsoclonus-myoclonus ataxia-like syndrome*
TPPI (or ***CLN2***)	Tripeptidyl peptidase 1 (TPP1)	**Classic late infantile** Juvenile, protracted, *SCAR7*
CLN5	CLN5 protein	**Late infantile** Juvenile, protracted, adult
CLN6	CLN6 protein	**Late infantile** Protracted **Adult type A Kufs** (recessive)
MFSD8 (or ***CLN7***)	MFSD8	**Late infantile** Protracted
CLN8	CLN8 protein	**Late infantile** Protracted **Northern epilepsy** (EPMR)
CLN3	CLN3 protein	**Classic juvenile** Protracted, *retinitis pigmentosa, autophagic vacuolar myopathy*
ATP13A2 (or ***CLN12***)	ATPase	**Juvenile** *Kufor-Rakeb syndrome*
DNAJC5 (or ***CLN4***)	Cysteine-string protein alpha (CSPα)	**Adult type A Kufs (dominant)**
CTSF (or ***CLN13***)	Cathepsin F	**Adult type B Kufs (recessive)**
GRN (or ***CLN11***)	Progranulin	**Adult** *Frontotemporal lobar dementia (heterozygous)*

typed hand movements may be present. Death takes place in the first decade of life. Even though mutations in *PPT1* are mainly responsible for this classic infantile NCL, later-onset forms (juvenile, adult) have also been described, probably due to less severe mutations.

Variants due to another gene More recently, mutations have been reported in *KCTD7* (now *CLN14*) in patients with infantile-onset progressive myoclonic epilepsy (PME), vision loss, cognitive and motor regression and premature death. *KCTD7* mutations have also been described in another phenotype called opsoclonus myoclonus ataxia syndrome (OMS) associating acute onset of myoclonus and ataxia with abnormal opsoclonus-like eye movements [139].

Classic Late Infantile Neuronal Ceroid Lipofuscinosis (LINCL, Jansky-Bielschowsky) Linked to *TPP1* (*CLN2*)

Children may be initially referred for delayed speech. Seizures, which may be of any type (partial, generalized tonic-clonic, absences), occur between 2 and 4 years of age. Ataxia, myoclonus and developmental regression become apparent, followed by a gradual decline of visual ability culminating in blindness by 5 or 6 years. Death happens in middle childhood after a bedridden stage. Besides this classic LINCL, mutations in *TPP1* have also been involved in atypical phenotypes with delayed onset and slower progression. Moreover, mutations in *TPP1* have been reported in autosomal recessive spinocerebellar ataxia 7 (SCAR7). Patients showed ataxia, but neither visual abnormalities, nor epilepsy and the disease is slowly progressive until old age.

Variants due to other genes Variants with similar or later onset, or delayed evolution compared to the classic late infantile form have been described. The Northern epilepsy or progressive epilepsy with mental retardation (EPMR) linked to *CLN8* is characterised by tonic-clonic seizures occurring between 5 and 10 years. Mental deterioration is observed 2 to 5 years after the onset of epilepsy. Vision problems are rare.

Some patients are living well over 40 years. Mutations in *CLN8* have also been reported in a subset of late-infantile patients from Turkish consanguineous families. A variant involving *CLN5* is common in Finland even if other patients have now been described in other countries. It usually begins around 4.5–6 years by clumsiness and difficulties in concentration. Visual impairment, ataxia and epilepsy appear a few years later. Life expectancy is between 13 and 35 years. Two additional genes are commonly involved in late-infantile variants presenting a clinical pattern close to the CLN2 disease. Mutations in *CLN6* are mainly seen in patients originating from South Europe, Indian subcontinent and South America. *CLN7* (*MFSD8)* has been initially involved in Turkish patients with LINCL, but abnormalities in this gene have now been reported in patients from different countries [139].

■ Classic Juvenile Neuronal Ceroid Lipofuscinosis (JNCL, Batten or Spielmeyer-Vogt) Linked to *CLN3*

The onset is between 4 and 10 years. Visual failure is usually the first clinical sign and it results in total blindness in 2–3 years. Seizures appear between 5 and 18 years, and predominant types are generalized tonic-clonic, myoclonic or partial seizures. Speech becomes dysarthric and echolalia is frequent. Many patients develop signs of parkinsonism. Mental capacity is progressively altered and dementia becomes evident in several years. Behavioural problems with aggressiveness may occur. Most patients live until the late teens or early/late 20s. A protracted atypical phenotype has recently been reported in patients showing a rapid visual failure followed 20 years later by seizures, hypertrophic cardiomyopathy, the presence of autophagic vacuoles in muscle biopsy and only mild cognitive impairment after 40 years of evolution.

Variant due to another gene Mutations in *ATP13A2* (now *CLN12*) have been associated with a NCL juvenile variant showing learning difficulties around 8 years, followed by unsteady gait, myoclonus, mood disturbance, and extrapyramidal signs such as akinesia, rigidity and dysarthric speech. It is important to note that *ATP13A2* is a known cause of Kufor-Rakeb syndrome which is a rare parkinsonian syndrome with juvenile onset marked by dementia, supranuclear palsy and pyramidal signs [139].

■ Classic Adult Neuronal Ceroid Lipofuscinosis (ANCL, Kufs)

Symptoms usually start around age 30 years, but onset during adolescence or late adulthood has been reported. Kufs disease is usually inherited as an autosomal recessive trait, but a rare dominant form (sometimes called Parry disease) also exists. Classically, two major forms of Kufs disease have been delineated. Type A is characterised by progressive myoclonic epilepsy while type B is marked by dementia and a diversity of motor signs. Retinal vision is generally preserved. During years, the genes involved in these forms have remained uncharacterised even though mutations have been found in *PPT1* in some patients. More recently, *CLN6* has been reported to be a major gene in recessive type A Kufs disease and the dominant form has been linked to *DNAJC5* (now called *CLN4*) encoding cysteine-string protein alpha (CSPα). Causal abnormalities have also been found in *CTSF* (or *CLN13*) encoding cathepsin F in patients with type B Kufs disease.

Variant due to another gene Mutations have been reported in *GRN* (or *CLN11*) encoding progranulin in siblings with rapidly progressive visual failure around 20 years, myoclonic seizures, cerebellar ataxia and early cognitive deterioration. Unexpectedly, these patients were homozygous for a *GRN* mutation, while heterozygous mutations in the same gene are a major cause of frontotemporal lobar dementia. These two diseases significantly differ by their age of onset and neuropathology [139].

■ Congenital Form

This rare form presents with microcephaly and seizures at birth, resulting in death within the first days of life. Mutations in *CTSD* (or *CLN10*) have been found in some patients, but other causative genes probably remain to be characterised [139].

38.4.2 Metabolic Derangement

Ceroid lipofuscinoses are characterised by the accumulation of autofluorescent ceroid lipopigments, mainly in neural tissues. They show different ultrastructural patterns, such as granular, curvilinear or fingerprint profiles [140]. The main components of this storage material are either saposins A and D in infantile forms, or subunit c of mitochondrial ATP synthase (SCMAS) in late infantile and juvenile forms. They are probably not disease-specific substrates, but secondary markers. NCL proteins are mainly localized in the lysosome (CLN1, CLN2, CLN3, CLN5, CLN7, CLN10, CLN12, CLN13), but also in the endoplasmic reticulum (CLN6, CLN8) or in the cytosol in association with vesicular membranes (CLN4, CLN14). Five of them are soluble proteins: palmitoyl protein thioesterase 1 (CLN1), tripeptidyl peptidase 1 (CLN2), cathepsin D (CTSD), cathepsin F (CTSF) and CLN5. Others are transmembrane proteins (CLN3, CLN7, CLN12), the function of which is badly understood. For a review, see [141]. Briefly, CLN1 is involved in the degradation of S-fatty acylated proteins and it possibly regulates exo- and endocytosis in the neuronal presynaptic area. CLN2 is a serine protease which removes tripeptides from proteins facilitating their degradation in lysosomes. It is likely involved in macroautophagy and TNFα-induced apoptosis. Cathepsin D is an aspartyl endopeptidase which has been related to apoptosis and autophagy while cathepsin F is a cysteine protease recently associated to proteasome degradation and autophagy. Function of CLN5 is still unclear. CLN3 was shown to affect lysosomal pH regulation, autophagy, proliferation and apoptosis. CLN6 has been associated with autophagy, regulation of pH, endocytosis, and biometal metabolism. CLN8 (member of a superfamily including TRAM and Lag1) could participate in regulation of sphingolipid metabolism. CLN7/MFSD8 belongs to the major

facilitator superfamily (MFS), and is therefore likely to transport small (still unidentified) molecules across membranes. Progranulin (CLN11) plays important roles in inflammation or tumorigenesis, and could be involved in autophagy and lysosomal homeostasis. ATP13A2 (CLN12) is a lysosomal transmembrane protein which belongs to the P-type ATPases family; its possible role is to protect against α-synuclein toxicity. CLN14 (KCTD7) probably affects neuronal excitability by regulating K+ conductance in neurons. CSPα, altered in the rare dominant adult form, is a molecular co-chaperone with Hsc70, potentially involved in synaptic vesicle exocytosis and endocytosis.

38.4.3 Genetics

NCLs are usually inherited in an autosomal recessive manner (except an adult form which is dominantly transmitted). They result from mutations in the thirteen known genes encoding the various NCL proteins [139] (Table 38.2). Numerous *PPT1* (*CLN1*) mutations have been reported, but p.Arg122Trp and p.Arg151* are common in Finnish and non-Finnish patients, respectively. Two mutations are common in *TPPI* (*CLN2*): c.509-1G>C and p.Arg208*, but more than 100 private mutations have now been described. For *CLN3*, a 1 kb deletion (c.461-280_677+382del966) is particularly frequent (80-90% of alleles). Concerning *CLN5*, p.Tyr392* is frequent in the Finnish population, but different mutations have been found in other countries. Northern epilepsy is mainly due to the p.Arg24Gly mutation, but other *CLN8* abnormalities have been described in patients presenting with late infantile variants. Numerous mutations have been reported in the other NCL genes characterised to date and the majority of them are private. Details are given in the NCL Mutation Database (http://www.ucl.ac.uk/ncl/).

38.4.4 Diagnostic Tests

Electrophysiological studies are helpful to establish the diagnosis of NCLs. Electroretinogram (ERG) is generally diminished at presentation and it becomes rapidly extinguished. In INCL, the first abnormality in the electroencephalogram (EEG) is the disappearance of eye opening/closing reaction, followed by a loss of sleep spindles. Then, EEG becomes rapidly flat. In LINCL, an occipital photosensitive response to photic stimulation at 1–2 Hz with eyes open is present. MRI shows progressive brain atrophy, particularly severe in INCL, sometimes beginning on cerebellum in other forms.

Vacuolated lymphocytes are only present in the juvenile form (*CLN3*). Electron microscopy (EM) on tissue biopsies – usually skin – shows the presence of pathological inclusions. Granular osmiophilic deposits (GROD) are mainly found in early forms involving *CLN1* or *CLN10/CTSD*. Curvilinear (CV) profiles are present in the classic LINCL (*CLN2*) and in the variant form linked to *CLN7*, while fingerprints (FP) are common in JNCL (*CLN3*). Mixed inclusions diversely associating GROD, CV and FP are found in LINCL variants (*CLN5, CLN6, CLN7, CLN8*) or in adult forms. EM is essential to confirm the diagnosis of NCL [140].

For the *CLN1* and *CLN2* loci, diagnosis is established by measuring respectively the palmitoyl protein thioesterase 1 and the tripeptidyl peptidase 1 activities, either on leukocytes or cultured fibroblasts, using specific artificial fluorogenic substrates. The disease-causing mutations are then characterised by complete sequencing of the corresponding genes [142]. The diagnosis strategy is similar for *CTSD* encoding cathepsin D (rare cases). For all the other genes, complete sequencing is performed directly, except for *CLN3* where it can be preceded by the rapid detection of the common 1 kb deletion [143]. Prenatal diagnosis is possible using specific enzymatic assay and/or detection of the mutation(s) previously determined in the index case. It is important to note that diagnosis of NCLs will soon benefit from the development of next generation sequencing (NGS). They will be diagnosed more rapidly by using gene panels specifically dedicated to these diseases or focused on pathologies (such as myoclonic epilepsies, ….) sharing clinical features with NCLs.

38.4.5 Treatment and Prognosis

Among symptomatic treatments, antiepileptic drugs need to be selected with caution. Lamotrigine is usually efficient on seizures. Levetiracetam may also be beneficial, but carbamazepine and phenytoin can worsen the symptoms. Diazepines should be useful on seizures, anxiety, and sleep disturbances. Gastrostomy is used to maintain nutritional status in the late stages of the disease. Specific therapies are in development for NCL and some of them are entering the clinic (http://clinicaltrials.gov/) [144]. In CLN2, two clinical trials are ongoing: ERT with intrathecal administration of recombinant TPP1, following demonstration of its efficacy in murine and canine models; and gene transfer with direct intracerebral administration of an AAV10 encoding *TPP1*. In CLN1 patients, a treatment combining cysteamine bitartrate and N-acetylcysteine was able to deplete GROD in peripheral leukocytes, but did not significantly change the course of the disease. As autoantibodies were found in the brain of juvenile NCL, a treatment based on mycofenolate mofetil was tested in mice, and demonstrated its capacity to decrease neuroinflammation and to protect neurons. A clinical trial is now ongoing in patients with a juvenile form involving *CLN3*. Several other experimental approaches, including neural stem cells, are under investigation [145].

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