

### 22.1 Introduction

Sphingolipids are essential constituents of cell membrane. They consist of a hydrophilic complex carbohydrate chain and a hydrophobic ceramide. In ceramide, an amino alcohol (sphingosin) is acylated with a long chain fatty acid through an amide linkage. All the sphingolipids are distinguished by their different polar groups at C-1. Many lysosomal enzymes are involved in the stepwise degradation of the sphingolipids. A genetic defect of one of these enzymes leads to disorders that are characterized by progressive storage in affected organs and functional impairment. The sphingolipidoses Gaucher disease and Niemann-Pick disease A and B are described in Chap. 19.14.

In Fabry disease, glycolipids with terminal  $\alpha$ -galactosyl moieties, mainly ceramidtrihexoside (globotriaosylceramide, Fig. 22.1), accumulate throughout the body, particularly in the skin, kidneys, nervous system, eyes and heart. Progressive accumulation of these glycolipids is caused by an inherited deficiency of the lysosomal hydrolase  $\alpha$ -galactosidase A. The gene encoding this enzyme is localized and physically mapped to the chromosomal region Xq22.1. In Fabry disease, crises of severe pain in the extremities (acroparesthesias), hypohidrosis, corneal opacities and dysfunction of several organs (kidney, brain, heart) are the leading symptoms. Females may have the same symptoms as males, but to a more variable degree. The variable manifestations seen in hemizygotes can be explained by the Lyon hypothesis. It predicts that in X-linked diseases the carriers are a mosaic of normal and mutant cells in varying proportions, and hence have variable expression. As in Gaucher's disease, also in Fabry disease, enzyme replacement therapy may be available in the future [1].

In the last step of sphingolipid degradation, ceramide is split into sphingosin and a long-chain fatty acid by the enzyme ceramidase. The genetic defect of this enzyme leads to Farber's disease, a storage disorder with onset in early childhood. In most cases, death occurs in the early years of life, but later-onset types with variable involvement of the central nervous system have also been observed.

The glycolipid cerebroside-3-sulfate (sulfatide), that represents an essential component of myelin sheaths of the nervous system, is degraded by the enzyme arylsulfatase A. In metachromatic leukodystrophy a deficiency of this enzyme leads to the accumulation of sulfatide in various organs, mainly affecting the central nervous system. The first signs of progressive demyelination are gait disturbances, and later affected patients develop spastic tetraparesis, seizures and dementia. In late onset forms, psychiatric symptoms precede the neurological abnormalities. Deficiency of arylsulfatase A has been observed also in healthy individuals. This so-called “pseudodeficiency” is caused by a mutation in a polyadenylation signal that allows only the synthesis of about 10% of arylsulfatase A compared to the normal allele.

In some patients, exhibiting symptoms of the juvenile type of metachromatic leukodystrophy, the disease is caused by a defect in saposin B, an activator that is necessary for full arylsulfatase activity [2].

For treatment, some patients with metachromatic leukodystrophy have had bone marrow transplantations performed. This procedure may slow the progression of the disease [3].

Not only glycosaminoglycans, oligosaccharides and sphingolipids, but also peptides and proteins are degraded in lysosomes. A defect of one of the enzymes that is involved in protein catabolism leads to multiple forms of a neurodegenerative disorder that are known as Batten’s disease (neuronal ceroid lipofuscinosis). All the types of Batten’s disease are characterized by progressive blindness and dementia. Initially, the various forms of Batten’s disease were classified according to age of onset, clinical phenotype and ultrastructural characterization of the storage material. Differences in the ultrastructural appearance of neurons and other cell types (e.g. granular osmiophilic deposits, curvilinear profiles, fingerprint bodies) have been the bases for both diagnosis as well as genetic research. Recently, in some of the several subtypes the underlying metabolic defect has been elucidated enabling the diagnosis to be made by enzymatic (and genomic) analysis [4]. Rapid loss of vision, psychomotor deterioration and seizures are the leading symptoms in infantile neuronal ceroid lipofuscinosis (INCL) where the activity of palmitoyl protein thioesterase 1 is lacking (Fig. 22.2). A deficiency of tripeptidyl peptidase 1 leads to late infantile neuronal ceroid lipofuscinosis (LINCL). In LINCL the storage material consists predominantly of subunit C, a hydrophobic protein that is normally found as an intrinsic inner membrane component of the ATP synthase complex.

A defect of a membrane-bound protein (CLN3 protein) has been found in juvenile neuronal ceroid lipofuscinosis. The function of CLN3 protein has not yet been elucidated.

Patients with juvenile neuronal ceroid lipofuscinosis show symptoms similar to those of the late infantile type (LINCL), but the progression is slower. In their twenties, affected individuals experience signs of parkinsonism, behavioral problems and psychological abnormalities.

In addition to the types mentioned in this chapter, there are at least five more groups of neuronal ceroid lipofuscinosis where the biochemical abnormality has not been determined although the genes linked to the disorders have been identified [5].

After degradation of glycoproteins, glycosaminoglycans and glycolipids, the acid sugars have to be transported out of the lysosomal compartment. Removal of these small molecules is facilitated by transport systems that have a broad substrate specificity (Fig. 22.3). The mammalian carrier responsible for the transport of N-acetylneuraminic acid (=sialic acid) also recognizes structurally different types of organic anions, like lactate, glucuronic and hexuronic acid. A genetic defect of this anion transporter is responsible for the sialic acid storage disorders [6], that present either as a severe infantile form (ISSD) or as a slowly progressive adult form which is unusually frequent in Finland (Salla disease). As a consequence of the transporter defect, urinary excretion of free sialic acid is increased. In 13 of 21 cases fetal/neonatal ascites or hydrops fetalis was the mode of presentation [7].

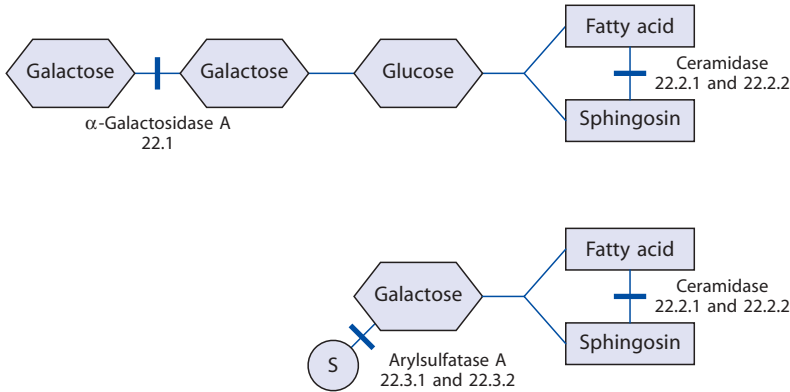
The sialic acid storage disorder has to be distinguished from the metabolic disease sialuria, that results from mutations in the gene encoding UDP-N-acetylglucosamine-2-epimerase (UDP-GlcNac-2-epimerase): In the first step of N-acetylneuraminic acid synthesis, UDP-N-acetylglucosamine is converted to N-acetylmannosamine and UDP by the enzyme UDP-GlcNac-2-epimerase. This enzyme is inhibited by CMP-N-acetylneuraminic acid, the activated sialic acid donor. Failure of this feedback mechanism due to mutations in the UDP-GlcNac-2-epimerase gene has been determined to be the cause of sialuria [8]. Patients with sialuria, who excrete massive amounts of free sialic acid in their urine (ranging up to several grams a day), show symptoms commonly recognized in storage disorders such as variable degrees of developmental delay, hepatomegaly and mild coarseness of facial features. Skeletal abnormalities (dysostosis multiplex) and seizures have also been observed [9].

# 22.2 Nomenclature

No.	Disorder	Enzyme defect	Chromosome localization	MIM <sup>a</sup>
22.1	Fabry disease	$\alpha$ -Galactosidase A	Xq22	301500
22.2.1	Farber disease (classical type)	Ceramidase	8p22-p21.3	228000
22.2.2	Farber disease (intermediate and mild type)	Ceramidase	8p22-p21.3	228000
22.3.1	Metachromatic leukodystrophy, infantile form	Arylsulfatase A	22q13.31-qter	250100
22.3.2	Metachromatic leukodystrophy, juvenile form	Arylsulfatase A	22q13.31-qter	250100
22.3.3	Metachromatic leukodystrophy (SAP B defect)	Saposin B	10q21.1	249900
22.4.1	Infantile neuronal ceroid lipofuscinosis (CLN1)	Palmitoyl protein thio-esterase 1 (PPT1)	1p32	256730
22.4.2	Late infantile neuronal ceroid lipofuscinosis (CLN2)	Tripeptidyl peptidase 1	11p15.5	204500
22.4.3	Juvenile neuronal ceroid lipofuscinosis (CLN3)	Membrane protein	16p12.1	204200
22.5.1	Sialic acid storage disorder (infantile form)	Sialin (specific transporter)	6q14-q15	604322 (#269920)*
22.5.2	Sialic acid storage disorder (Salla disease)	Sialin (specific transporter)	6q14-q15	604369 (#269920)*
22.6	Sialuria	UDP-N-acetylglucosamine 2-epimerase	9p12-p11	269921

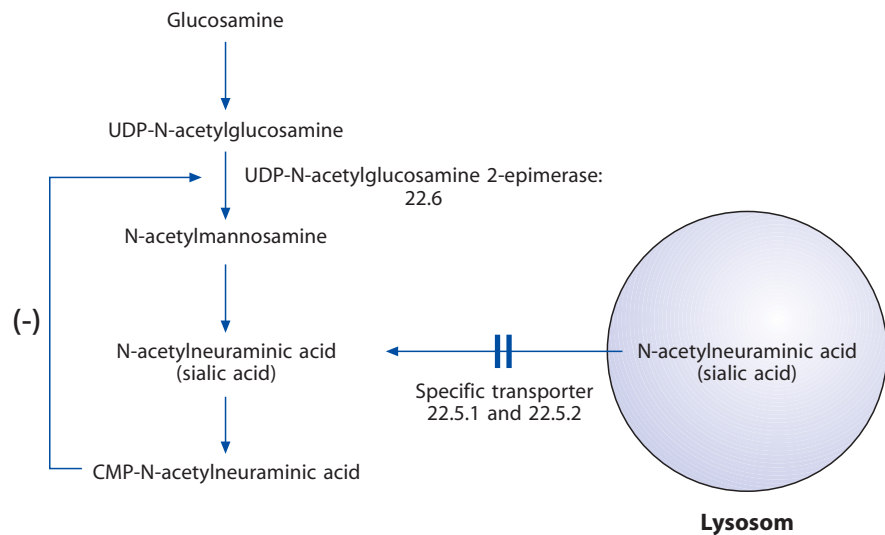
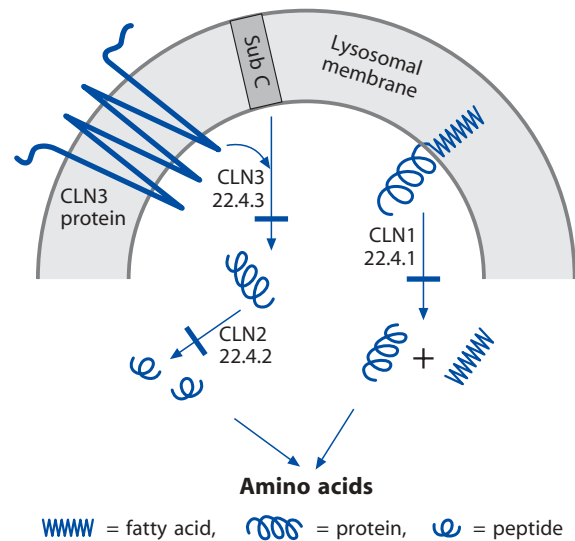
<sup>a</sup> A number sign (#) is used with this entry because of both the infantile (MIM 604322) and Finnish (Salla disease, MIM 604369) forms of sialuria are due to mutation in the SLC17A5 gene (MIM 604322).

# 22.3 Metabolic Pathways



**Fig. 22.1.** Degradation of sphingolipids. The numbers in bold indicate the diseases listed in Sect. 22.2

**Fig. 22.2.** Lysosomal catabolism of membrane-associated/hydrophobic proteins. In infantile neuronal ceroid lipofuscinosis (CLN1) palmitoyl protein thioesterase is deficient (22.4.1). Late infantile neuronal lipofuscinosis (CLN2) is due to a defect in tripeptidyl peptidase I (22.4.2). Sub C = subunit C of ATP synthase complex. CLN3 protein = membrane protein of unknown function. It probably contributes to the disposal of organella membranes, it is defective in juvenile neuronal ceroid lipofuscinosis (CLN3, 22.4.3)



**Fig. 22.3.** Metabolism of free N-acetylneuraminic acid (= sialic acid): UDP-acetylglucosamine 2-epimerase is inhibited by the activated sialic acid donor, CMP-N-acetylneuraminic acid, which also provides N-acetylneuraminic acid for glycoconjugate synthesis. In sialuria (22.6) loss of feedback inhibition leads to overproduction of sialic acid. In sialic acid storage disorders (22.5.1 and 22.5.2) N-acetylneuraminic acid accumulates in the lysosomes due to a defect of a specific transporter (sialin)

## 22.4 Signs and Symptoms

System	Signs/symptoms	Disorders		
		22.1 Fabry disease	22.2.1 Farber disease, classical type	22.2.2 Farber disease, intermediate and mild type
Clinical course	Age at onset	<10	<4 months	<20 months
	Age at death	>40	<1 year	<20 years
	Characteristic clinical findings	Angiokeratoma Cornea verticillata Acroparesthesia	Joint swelling Hoarseness	Joint swelling Hoarseness
		±		
Facies	Coarse facial features	±		
Skeleton	Joint swelling	+	+++	+++
	Joint thickening		+++	+++
	Joint deformity		+++	+++
	Contractures	±	+++	+++
Eye	Corneal opacities	+++	±	±
	Cornea verticillata	+++		
	Cataract		±	
	Macular cherry red spot		±	
Ear	Tinnitus	+++		
Central nervous system	Stroke	±		
	Mental retardation	±	+	±
	Neurological deterioration	±	+	±
	Dementia	±	+	±
Cardiac	Valvular thickening	+	+	±
	Cardiomegaly	++	+	±
Kidney	Renal insufficiency	+++		
Spleen	Splenomegaly		±	±
Liver	Hepatomegaly		±	±
GI	Swallowing difficulties		++	±
	Diarrhea	+		
Dermatologic	Angiokeratoma	+++		
	Hypohidrosis	+++		
Special laboratory	Lipid crystals in urine sediment (Maltese cross)	+++		

System	Signs/symptoms	Disorders		
		22.3.1 Metachromatic leukodystrophy, infantile	22.3.2 Metachromatic leukodystrophy, juvenile	22.3.3 Metachromatic leukodystrophy (SAP B deficiency)
Clinical course	Age at onset	<2 years	<10 years	<2 years
	Age at death	<10 years	<20 year	<20 years
	Characteristic clinical findings	Spastic tetraplegia	Spastic tetraplegia	Spastic tetraplegia
		Optic atrophy	Optic atrophy	Optic atrophy
		Gallbladder stones	Gallbladder stones	
Skeleton	Genu recurvatum	±		
Eye	Optic atrophy	++	+	±
	Greyish colour of macula	++		
	Nystagmus	++	±	
Peripheral nervous system	Neuropathy	+++	++	++
Central nervous system	Behaviour abnormality	±	++	++
	Ataxia	+++	+	±
	Spastic tetraplegia	+++	+++	+++
	Seizures	±	++	+++
	Progressive mental retardation	+++	+++	+++
Kidney	Renal tubular acidosis	±		
Spleen	Splenomegaly			±
Liver	Hepatomegaly			±
GI	Gallbladder papilloma	±	±	
	Gallbladder stones	+	+	
	Gastrointestinal hemorrhage	±		
	Swallowing difficulties	+++	++	++
	Increased sulfatide excretion	+++	+++	+++
Special laboratory	Increased spinal fluid protein	+++	+++	+
	Nerve conduction velocity reduced	+++	+++	+++

System	Signs/symptoms	Disorders		
		22.4.1 Neuronal ceroid-lipofuscinosis, infantile	22.4.2 Neuronal ceroid-lipofuscinosis, late infantile	22.4.3 Neuronal ceroid-lipofuscinosis, juvenile
Clinical course	Age at onset	<1 year	<4 years	<7 years
	Age at death	<10 years	<10 years	<30 years
	Characteristic clinical findings	Visual failure	Visual failure	Visual failure
		Seizures	Seizures	Psychotic symptoms
		Mental retardation	Mental retardation	Parkinsonism
Skeleton	Microcephaly	+	+	
Eye	Optic atrophy	+++	+	+++
	Retina degeneration	+++	+	+++
	Cataract			+
	Brownish colour of macula	+++		
	ERG abnormality	+++	+++	+++
Central nervous system	Muscular hypotonia	+++	±	±
	Ataxia	++	++	±
	Myoclonus	++	++	±
	Seizures	+++	+++	+
	Progressive mental retardation	+++	+++	++
Cardiac	ECG abnormality			+++
Dermatologic	Hirsutism			+
	Hyperpigmentation			+
EM findings (nerve cells)	Granular osmiophilic deposits	+++		
	Curvilinear profiles		+++	
	Rectilinear profiles			+++



System	Signs/symptoms	Disorders		
		22.5.1 Sialic acid storage disease, infantile form	22.5.2 Sialic acid storage disease, adult form, Salla disease	22.6 Sialuria
Clinical course	Age at onset	from birth	>6 months	<2 years
	Age at death	<4 years	normal life expectancy	
	Characteristic clinical findings	Coarse facial features	Ataxia	Mental retardation
		Mental retardation	Dysarthria	Cardiomegaly
		Fair skin	Mental retardation	Hepatosplenomegaly
Facies	Coarse facial features	+++	+	+
Skeleton	Dysostosis multiplex	+++		+
	Thickened calvarium		+	
Eye	Pale fundus	+		
	Nystagmus		+	
Central nervous system	Spastic tetraplegia	±	++	+
	Seizures	+++	+	+
Cardiac	Cardiomegaly	++		+++
Kidney	Nephrotic syndrome	++		
Spleen	Splenomegaly	+++		+++
Liver	Hepatomegaly	+++		+++
GI	Ascites	±		
Unique clinical findings	Hydrops fetalis	±		
Laboratory	Urine excretion of free N-acetylneuraminic acid	+++	+	+++

## 22.5 Laboratory Diagnosis

Disorder	Enzyme defect	Material		Method
		Postnatal diagnosis	Prenatal diagnosis	
22.1	$\alpha$ -Galactosidase A	WBC, P, FB	CV, AFC	Enzyme assay <sup>a</sup>
22.2.1 and 22.2.2	Ceramidase	FB	AFC	Enzyme assay <sup>a</sup>
22.3.1 and 22.3.2	Arylsulfatase A	WBC, FB	CV, AFC	Enzyme assay <sup>a</sup>
22.3.3	Saposin B	FB	AFC	Enzyme assay <sup>b</sup>
22.4.1	Palmitoyl protein thioesterase 1 (PPT1)	WBC, FB	CV, AFC	Enzyme assay <sup>b</sup> , ultrastructural investigation <sup>c</sup>
22.4.2	Tripeptidyl peptidase I	WBC, LYM	AFC	Mutation analysis, ultrastructural investigation <sup>c</sup>
22.4.3	Membrane protein	WBC, LYM	AFC	Mutation analysis, ultrastructural investigation <sup>c</sup>
22.5.1 and 22.5.2	Sialin (specific transporter)	U, FB	AFC	Measurement of free sialic acid in urine and fibroblasts
22.6	UDP-N-acetylglucosamine 2-epimerase	U, FB	AFC	Measurement of free sialic acid in urine and fibroblasts, enzyme assay <sup>b</sup>

<sup>a</sup> The diagnosis of these groups of disorders relies upon the demonstration of a profound deficiency of specific enzymatic activities, assayed in the appropriate material. Since assay conditions are very variable in different laboratories, reference values are not given here.

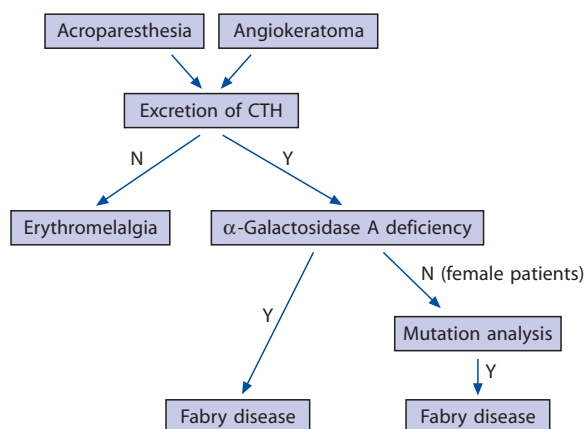
<sup>b</sup> These enzyme defects can be detected in only a few laboratories specialized in these rare disorders.

<sup>c</sup> In neuronal ceroid lipofuscinosis the diagnosis may be based on the demonstration of a characteristic ultrastructural pattern in lymphocytes and/or nerve cells alone. The diagnosis can be confirmed by mutation analysis.

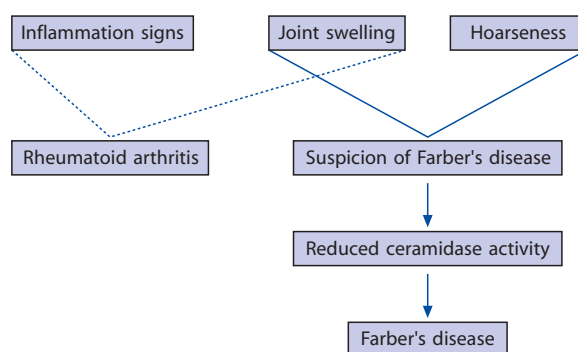
## 22.6 Diagnostic Flow Charts

**Fig. 22.4.** Clinical approach to the diagnosis of Fabry disease. In affected females,  $\alpha$ -galactosidase activity is often in the normal range. In those cases, the diagnosis has to be confirmed by mutation analysis.

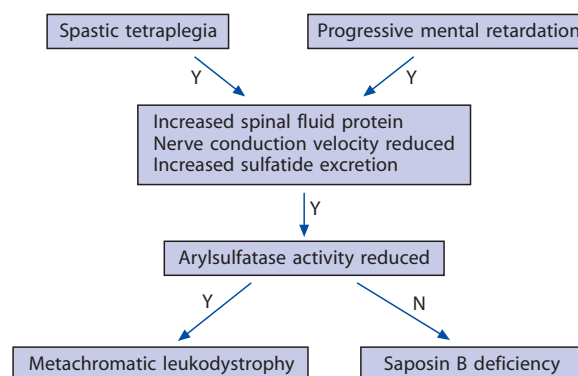
CTH = Ceramidtrihexoside

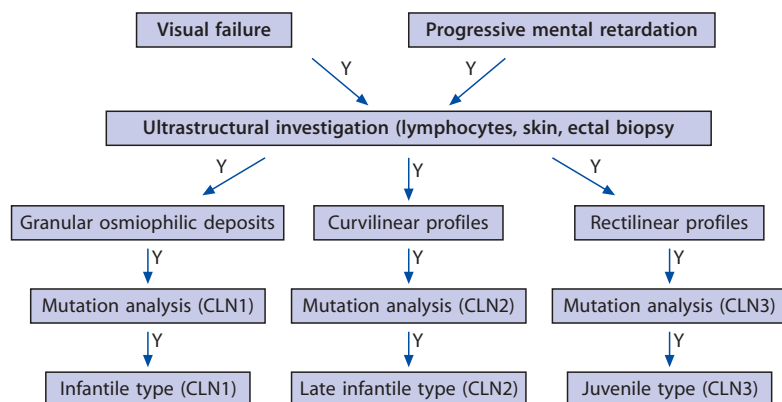


**Fig. 22.5.** Joint swelling and hoarseness are the leading symptoms in Farber's disease

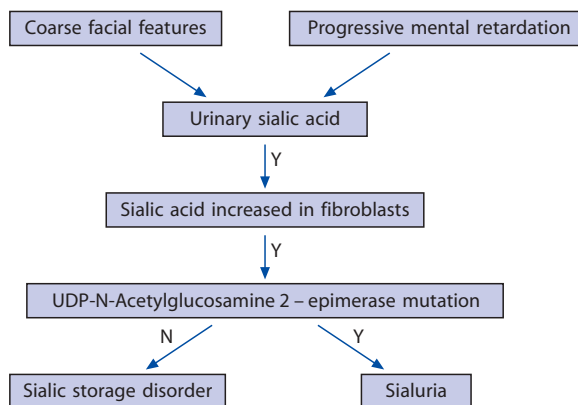


**Fig. 22.6.** As there are healthy individuals with deficient arylsulfatase A activity, the diagnosis of metachromatic leukodystrophy cannot be made by enzyme analysis alone. The diagnosis must be confirmed by the detection of increased protein concentration in spinal fluid and reduced nerve conduction velocity. Increased sulfatide excretion is also found in Saposin B deficiency





**Fig. 22.7.** In the several types of neuronal ceroid lipofuscinosis, the diagnosis is based on characteristic clinical features (visual loss and progressive mental retardation) and on the demonstration of ultrastructural changes in several cell types (nerve cells, lymphocytes). Mutation analysis enables the definitive diagnosis



**Fig. 22.8.** Coarse facial features and progressive mental retardation are characteristic symptoms in both, sialic storage disorder and sialuria. Differentiation is possible only by measuring UDP-acetylglucosamine 2-epimerase, that is mutated in sialuria

## 22.7 Summary

Angiokeratoma, hypohidrosis and renal dysfunction are the primary symptoms of Fabry disease ( $\alpha$ -galactosidase deficiency), an X-linked storage disorder with clinical manifestation also in the female carriers. Farber's disease (ceramidase deficiency) is characterized by painful joint swelling and hoarseness. In many patients mental retardation is also observed. In metachromatic leukodystrophy (arylsulfatase A or saposin B deficiency) progressive demyelination leads to severe neurodegenerative symptoms. In patients with progressive mental retardation combined with visual loss, one of the several types of Batten's disease (neuronal ceroid lipofuscinosis) has to be considered. That diagnosis is based on the detection of ultrastructural alterations in nerve cells and lymphocytes. In the sialic acid storage disorders (infantile and adult form) sialic acid is stored in the lysosomes because of a defect of a specific transporter. In sialuria there is an increase of sialic acid synthesis caused by a failure of a feedback mechanism. The sialic storage disorders and sialuria have in common clinical symptoms such as developmental delay and visceromegaly.

## References

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