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Sialic Acid Storage Disease and Related Disorders

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ABSTRACT

This paper gives an overview of the two sialic acid storage disorders, Salla disease and infantile sialic acid storage disease, and the related disorders cystinosis, sialuria, sialidosis, and galactosialidosis. Sialic acid storage disease and cystinosis are models for a deficient lysosomal transport of monosaccharides and amino acids, respectively. Several gene mutations leading to the production of the faulty membrane proteins sialin and cystinosin have been identified in recent years. Knowledge of the underlying pathophysiology is a prerequisite for future research projects, which will focus on the expression of the disease genes in living systems and the physical characterization of these proteins by X-ray crystallography and nuclear magnetic resonance spectroscopy.

INTRODUCTION

SIALIC ACID STORAGE DISORDERS (SASD) belong to the category of rare lysosomal storage diseases and can be divided into the adult-type Salla disease (SD) (OMIM 604369) and the more severe infantile sialic acid storage disease (ISSD; OMIM 269920). So far less than 150 cases, predominantly from Finland, have been reported. As in many other inborn errors of metabolism, the mode of inheritance is autosomal recessive. Patients with these progressive disorders show varying degrees of neurodevelopmental delay and learning difficulties. The storage of free sialic acid in the body and its excretion in the urine is increased up to 100 times. SASD are caused by a defective molecular transport mechanism, that leads to an accumulation of unbound sialic acid within the lysosomes (Gahl *et al.,* 1985).

Recently the gene responsible for the defect has been located on the long arm of chromosome 6. This gene encodes a transmembrane protein, sialin (OMIM 604322), which is a member of the major facilitator superfamily (MFS). The MFS comprises a large number of proteins involved in intra- and intercellular transfer of metabolites (Verheijen *et al.,* 1999). Sialidosis and galactosialidosis are characterized by intralysosomal storage of sialyl-oligosaccharides, which is caused by a deficiency of the enzyme sialidase. In contrast, sialuria is the result of an increased synthesis of sialic acids in the cytoplasm secondary to a lack of feedback inhibition of the rate-limiting enzyme (Fig. 1).

SIALIC ACID STORAGE DISORDERS

Salla disease

In 1978, Aula and colleagues described three adult brothers and their female cousin from an institution for disabled people in Finland whose clinical picture was suggestive of a new lysosomal storage disease (Aula *et al.,* 1978). Their signs and symptoms were coarse facial features, dysarthria, clumsiness, ataxia, and severe learning difficulties. The skull bones were thickened on radiography and the electroencephalogram was diffusely abnormal. The blood film revealed between 4% and 15% vacuolated lymphocytes, but the activities of eight lysosomal enzymes were normal. The concentration of total sialic acid in the urine was three to four times higher than in controls. On electron microscopy, large lysosomal storage vacuoles were detected in several types of skin cells, including Schwann cells, eccrine gland cells, and cultured fibroblasts (Aula *et al.,* 1979). All patients had an uneventful neonatal period. The first signs of delay were present at 1 year of age, and a significant deterioration was observed during the second decade. In view of the unknown etiology, the condition was named Salla disease (SD) after the region in northeastern Finland where the patients lived.

Using various chromatographic techniques, Renlund and coworkers examined urine specimens from 13 patients, who met the criteria for SD, and 24 healthy individuals. Gel chromatography revealed a characteristic sialic acid pattern in SD

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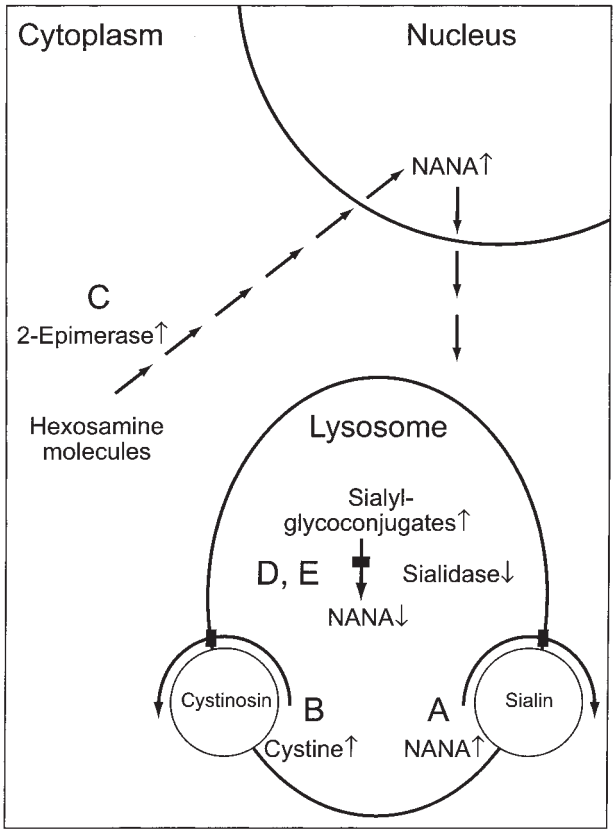

FIG. 1. Schematic representation of the metabolic pathways in: **A,** sialic acid storage disease; **B,** cystinosis; **C,** sialuria; **D,** sialidosis; and **E,** galactosialidosis (NANA, *N*-acetylneuraminic acid; 2-epimerase, uridine diphosphate *N*-acetylglucosamine 2-epimerase).

patients. One main peak (V) was analyzed further by combined gas-liquid chromatography/mass spectrometry and identified as *N*-acetylneuraminic acid (NANA or NeuAc). Urinary excretion of free (unbound) sialic acid ranged from 50.3 to 140.0 μg/mg creatinine in patients and 2.3–8.8 μg/mg in controls. The proportion of free to total sialic acid was increased about five-fold (Renlund *et al.,* 1979). The same authors collected data on 34 Finnish patients, who belonged to 23 partly interrelated families from the Salla district. They were between 2 years 9 months and 63 years old; 20 of them were male, and most had a normal birth history. Their intelligence quotient (IQ) did not exceed 40, and short stature was present in 18 affected adults (Renlund *et al.,* 1983a).

Radioactive labeling studies were performed on skin fibroblasts from normal individuals and from patients with SD. The results led to the conclusion that NANA is released from the lysosomes in mutant cells at a slower rate than from control cells (Renlund *et al.,* 1986a). Although SD occurs predominantly in Finland, sporadic cases from other countries have been reported. Wolburg-Buchholz *et al.* (1985) described 3 German siblings with a severe neurodegenerative disease leading to death in 2 of the patients. Morphological and biochemical findings were consistent with SD (Wolburg-Buchholz *et al.,* 1985). A 5-year old French boy was investigated for learning difficulties, speech delay, hyperactivity, and ataxia. Conjunctival biopsy showed vacuolated fibroblasts and thin-layer chromatography revealed high concentrations of free sialic acid in the urine (Echenne *et al.,* 1986).

It has been suggested that SD is underdiagnosed, because it lacks the typical features of other lysosomal storage disorders and usually becomes manifest in the second decade of life (Robinson *et al.,* 1997). Renlund and Aula monitored through amniocentesis 4 pregnant mothers from families at risk of SD. *N*-Acetylneuraminic acid in cultured amniocytes from one pregnancy was significantly raised compared to controls (2.6 nmol/mg protein vs. 0.5 nmol/mg protein). The infant developed hypotonia and nystagmus, and the diagnosis of SD was confirmed by skin biopsy (Renlund and Aula, 1987).

The involvement of the central nervous system in SD has been documented in several studies. Autopsy of 2 deceased patients revealed marked cerebral atrophy with loss of axons and myelin. The number of neurons in the cortex and the basal ganglia was reduced, and the remaining cells showed signs of degeneration (Autio-Harmainen *et al.,* 1988). Haataja and coworkers (1994a) performed magnetic resonance imaging (MRI) on 7 SD patients, who were between 11 months and 39 years old, and suffered from frequent seizures. They demonstrated defective white matter myelination, hypoplastic corpus callosum, and cortical and cerebellar atrophy. The MRI abnormalities correlated with the clinical severity (Haataja *et al.,* 1994a).

A Finnish research group studied the brain metabolism of nine subjects with SD using positron emission tomography (PET). The local cerebral metabolic rates for glucose were increased in the frontal and sensorimotor cortex, and the basal ganglia (Suhonen-Polvi *et al.,* 1999). In about 50% of SD patients, the peripheral nervous system is also affected. Varho and colleagues (2000) measured a decrease in motor nerve conduction velocities suggestive of a peripheral polyneuropathy. So far no specific treatment for patients with SD is available.

Infantile sialic acid storage disease

In 1982 Hancock *et al.* reported a male infant of German-Swedish origin, who presented at birth with severe ascites and hepatomegaly, and died at the age of 5 months. They found large amounts of NANA in brain, liver, and kidney tissues and linked this condition to SD. Studies on radioactively labeled cultured fibroblasts indicated the accumulation of free sialic acid in the lysosomes, but normal activities of the lysosomal enzymes. The concentration of free NeuAc in pathologic fibroblasts was 3.5 $\pm$ 0.7 μmol/gram fresh weight compared to 0.2 $\pm$ 0.04 μmol/gram in control fibroblasts (Hancock *et al.,* 1982, 1983). Tondeur and Stevenson and their colleagues delineated the infantile form of sialic acid storage disease further on the basis of 4 non-Finnish patients (Tondeur *et al.,* 1982; Stevenson *et al.,* 1983). The clinical features in these cases were sparse hair, coarse facies, hypopigmentation, hepatosplenomegaly, developmental delay, failure to thrive, and death in infancy. Vacuolated lymphocytes were present in blood and bone marrow smears, and the urinary excretion of unbound sialic acid was increased 20- to 60-fold.

Infantile sialic acid storage disease differs from SD insofar as its onset is earlier, the physical signs are more pro-

nounced, and it progresses much more rapidly. Unlike SD, ISSD is not limited to Finland, but has been observed in several patients from Europe, Northern America, and Australia (Cooper *et al.,* 1988; Clements *et al.,* 1988; Pueschel *et al.,* 1988; Levebre *et al.,* 1999; Sperl *et al.,* 1990). Lemyre *et al.* reviewed 27 cases of ISSD and listed as additional findings hydrops fetalis, cardiomegaly, nephrotic syndrome, and death at a mean age of 13.1 months (Lemyre *et al.,* 1999). Berra and colleagues performed extensive electron microscopical and biochemical studies on a female infant with ISSD, who died at the age of 3 months. Storage of free sialic acid was seen in brain, liver, spleen, kidneys, and cultured skin fibroblasts. While the tissue content of total cholesterol, phospholipids, and glycolipids was within normal limits, significant changes were noted in the distribution of complex membrane lipids (Berra *et al.,* 1995).

The underlying pathomechanism of ISSD is an impaired transport of free sialic acid and glucuronic acid (GlcUA) across the lysosomal membrane (Tietze *et al.,* 1989; Blom *et al.,* 1990). Vamos and co-authors (1986) diagnosed ISSD in a 17-week-old fetus using amniocentesis. The fetus was subsequently aborted, and ultrastructural examination revealed fibrillo-granular storage material in all cell types (Vamos *et al.,* 1986). It is also possible to diagnose ISSD morphologically and biochemically on chorion villus samples at 10 weeks gestation (Lake *et al.,* 1989). Only supportive treatment can be offered to children with ISSD.

Intermediate types

Some of the Finnish patients with SD and single cases from other parts of Europe presented in an atypical way that was more severe and included clinical findings usually only seen in ISSD. A 4-year-old boy of consanguinous Italian parents had mild dysmorphic features and hepatosplenomegaly at birth. During the first year of life, he required several admissions to hospital because of respiratory tract infections. His developmental milestones were delayed, his gait was broad based, and he was hypertonic. Numerous vacuolated cells were observed in blood, skin, and liver cells, and urinary free sialic acid was increased. The authors concluded that this case showed characteristics of both ISSD and SD, possibly due to allelic mutations (Baumkoetter *et al.,* 1985). Ylitalo and co-workers described 2 unrelated Swedish girls, who were initially thought to have ataxic and dyskinetic cerebral palsy, respectively. Their severe motor disability was associated with a normal early intellectual development. The concentration of free sialic acid in the urine was moderately high and storage lysosomes were present in Schwann cells and fibrocytes. They suggested, that these children had variants of SD (Ylitalo *et al.,* 1986).

Schleutker *et al.* (1995b) compared the different phenotypes of SASD and delineated an intermediate type in four non-Finnish children with severe psychomotor delay during the first decade. One of them also developed corneal opacities and ovoid deformation of the lumbar vertebral bodies (Fois *et al.,* 1987; Mancini *et al.,* 1992b; Schleutker *et al.,* 1995b). The number of reported patients with SASD worldwide is about 150. Therefore, it is likely that with increasing case reports the spectrum of these heterogeneous disorders will become more transparent.

RELATED DISORDERS

Cystinosis

Like SD and ISSD, cystinosis (OMIM 219800) is an autosomal recessive lysosomal storage disorder, which was first described by Abderhalden (1903). There are two main variants of cystinosis: the nephropathic type, which can be further subdivided depending on the age of onset; and the nonnephropathic, benign type. According to a French study, its overall incidence lies between 1 in 26,000 and 1 in 326,000 (Bois *et al.,* 1976).

Cystinosis is caused by a deficient lysosomal carrier system, that normally enables the amino acid cystine to leave the lysosome for further degradation. This results in deposition of cystine in various body tissues, particularly in kidneys, eyes, thyroid, pancreas, and gonads. Musculature and the nervous and gastrointestinal systems are, to a lesser extent, affected, as well. Infants with cystinosis are well at birth, but show symptoms of renal tubular acidosis (Fanconi syndrome) after 6 months. They have a reduced appetite and poor weight gain. Polyuria and polydipsia lead to recurrent dehydration and acidosis secondary to loss of water, electrolytes, and other small molecules. Later, the children develop photophobia and most of them require renal dialysis or a kidney transplant by the age of 10 years. The diagnosis is made by measuring the concentration of cystine in blood leukocytes or skin fibroblasts. Prenatal diagnosis using amniocytes and chorion villus specimens is also available.

The prognosis of cystinosis patients has greatly improved since the introduction of oral cysteamine treatment. Cysteamine lowers the cystine content of affected cells rapidly and delays the disease process significantly. After entering the lysosome, cysteamine forms a complex with one cysteine residue of the cystine molecule, and then exits the lysosome via an alternative pathway (Gahl *et al.,* 1995).

In 1995 the Cystinosis Collaborative Research Group investigated 43 cystinosis patients with 23 families to establish linkage between the gene for cystinosis and 328 microsatellite markers. They were able to assign the gene locus to a short segment on chromosome 17p, between the markers D17S1583 and D17S796 (McDowell *et al.,* 1995). Town and co-authors refined the critical region further and identified a new gene on chromosome 17p13 that is deleted in 33% of homozygous cystinosis patients. The latter CTNS gene encodes the lysosomal membrane protein "cystinosin," which consists of 367 amino acids and has six or seven predicted transmembrane domains (Town *et al.,* 1998).

Mutation screening and haplotype analysis of 34 cystinosis patients revealed 24 different mutations, the commonest being a 57-kb deletion. These mutations impair the function of the transport protein "cystinosin" to varying degrees and are responsible for the different phenotypic presentations (Attard *et al.,* 1999). Touchman *et al.* (2000) constructed a high-resolution physical map of the region containing the CTNS gene and detected a second gene, CARKL, in this region that encodes a carbohydrate kinase. Both genes are deleted in approximately half of the homozygous cystinosis patients (Touchman *et al.,* 2000).

Sialuria

Sialuria (OMIM 269921) was first reported in a 2.5-year-old French boy in 1968 (Fontaine *et al.,* 1968; Montreuil *et al.,*

1968). He presented with facial dysmorphism, skeletal abnormalities, failure to thrive, developmental delay, epilepsy, hepatomegaly, and metabolic acidosis. Thin-layer chromatography of his urine revealed an elevated daily excretion of 5.8 grams to 7.2 grams of NeuAc. Since then, only 3 further cases from Australia and America have been published (Wilcken *et al.,* 1987; Seppala *et al.,* 1991; Krasnewich *et al.,* 1993). Thomas and co-workers (1985) detected more than 40 times higher concentrations of free sialic acid in cultured skin fibroblasts from the original patient compared to control cells. Further biochemical studies by the same group provided evidence that the storage of NeuAc occurs in the cytoplasm rather than in the lysosomes (Thomas *et al.,* 1989).

The underlying metabolic defect in sialuria is the loss of feedback inhibition of the rate-limiting enzyme of sialic acid biosynthesis, uridine diphosphate N-acetylglucosamine 2-epimerase (UDP-GlcNAc 2-epimerase). The chemical reaction is normally controlled by cytidine monophosphate-*N*-acetylneuraminic acid (CMP-NeuAc). This leads to enhanced enzymatic activity and accumulation of NeuAc in the cytoplasm (Weiss *et al.,* 1989).

Recently the gene for UDP-GlcNAc 2-epimerase on chromosome 9p12 was successfully cloned, and 3 heterozygous mutations were identified suggesting a dominant mode of inheritance (Seppala *et al.,* 1999). One case of sialuria has yet to be classified. Palo and colleagues (1985) described an adult female of Finnish origin, who had mild learning difficulties, epilepsy, and sialuria, but no storage vacuoles on blood and biopsy samples. The daily amount of NeuAc in the urine was 0.5 gram, which is less than in the other sialuria patients, but similar to patients with sialic acid storage disorders (Palo *et al.,* 1985).

Sialidosis

Sialidosis (neuraminidase deficiency, OMIM 256550) is an autosomal recessive lysosomal storage disease affecting the degradation of glycoproteins. Two types of sialidosis are recognized. The milder form (type I) usually presents in the second decade with progressive visual impairment, generalized myoclonus, ataxia, and epilepsy. A characteristic finding is an ocular cherry-red spot. Sialidosis type II can be divided into 3 subgroups with descending severity (congenital, infantile, and juvenile). Physical signs include hydrops fetalis, coarse facies, hepatosplenomegaly, skeletal abnormalities, and delayed neurodevelopment (Thomas and Beaudet, 1995).

Both types are caused by a deficiency of the enzyme sialidase (α-neuraminidase), which removes terminal sialic acid molecules from oligosaccharides and glycoproteins. As a result, these compounds accumulate in various body tissues and are excreted in large amounts in the urine, where they can be identified by thin-layer chromatography (Strecker *et al.,* 1977). Sialidase activity can be also directly measured in amniotic fluid cells, skin fibroblasts, and leukocytes. Preliminary studies by Mueller and co-authors (1986) suggested that the gene encoding sialidase was located on the short arm of chromosome 10. In 1996, however, Bonten and colleagues (1996) isolated the cDNA for human α-neuraminidase and assigned the gene to the chromosome segment 6p21. Sialidase has a predicted molecular weight of 45 kD and consists of 415 amino acids (Bonten *et al.,* 1996). No curative treatment for patients with sialidosis is currently available.

Galactosialidosis

Galactosialidosis (neuraminidase deficiency with β-galactosidase deficiency, OMIM 256540) is a lysosomal storage disease closely related to sialidosis. One of the first patients with confirmed galactosialidosis was a 12-year-old Mexican boy, who displayed characteristics of the mucopolysaccharidoses, sphingolipidoses, and mucolipidoses, but could not be classified clearly. He had coarse facial features, delayed neurodevelopment, epilepsy, and cherry-red ocular spots. On electron microscopy, cytoplasmic vacuoles were seen in hepatic Kupffer cells and the activity of β-galactosidase was significantly decreased in skin biopsy specimens. Subsequently he was also found to be deficient for sialidase (Goldberg *et al.,* 1971).

Currently, three categories of galactosialidosis are recognized. Children with the early infantile form show hydrops fetalis, telangiectasias, organomegaly, skeletal abnormalities, and a shortened life span. The late infantile type has a milder presentation, with coarse facies, cherry-red foveae, dysostosis multiplex, valvular heart disease, and developmental delay. The majority of patients belong to the juvenile/adult type, which usually manifests in the second decade and predominantly occurs in consanguineous Japanese families. Typical findings are coarse facies, visual impairment, spinal abnormalities, and skin lesions. Neurological signs include myoclonus, seizures, ataxia, and learning difficulties.

The underlying pathomechanism of galactosialidosis is a defect in the lysosomal "protective protein"/cathepsin A (PPCA), which is related to the serine carboxypeptidases. PPCA is essential for the normal activity of α-neuraminidase and delays proteolysis of β-galactosidase. All three molecules form an enzyme complex. Mueller and co-authors (1986) concluded from their genetic complementation analysis that a gene on chromosome 20, which is necessary for the expression of sialidase, is nonfunctional in galactosialidosis fibroblasts. Further *in situ* hybridization studies assigned the gene locus for PPCA to chromosome 20q13.1. Zhou *et al.* (1996) investigated PPCA mRNA in 8 patients, aged 2.5 months to 48 years, and identified several different mutations that result in a decreased activity of PPCA in the lysosome. They observed a correlation between the level of PPCA and the severity of galactosialidosis (Zhou *et al.,* 1996).

The clinical signs of the disease are secondary to lysosomal storage of sialyloligosaccharides in multiple organs. The diagnosis is confirmed by a combined assay of the deficient enzymes on white blood cells or cultured skin fibroblasts, and demonstration of increased sialyloligosaccharides in the urine by thin-layer chromatography. The treatment for patients with galactosialidosis is symptomatic (D'Azzo *et al.,* 1995).

SIALIC ACID METABOLISM

N-*acetylneuraminic acid*

Sialic acids, also called nonulosaminic acids, comprise a group of 23 naturally occurring amino sugars with nine or more carbon atoms. The term "sialic acids" was first proposed by Blix *et al.* for all acylated neuraminic acids (Blix *et al.,* 1957). Due to their ability to form chemical bonds with peptides and carbohydrates, they are found as components of oligosaccha-

rides, polysaccharides, glycoproteins, and gangliosides (Zilliken *et al.,* 1958). Sialic acids have been purified and crystallized in substantial amounts from bovine and equine submaxillary glands. They can be determined qualitatively by the direct Ehrlich reaction and Bial's orcinol reaction (Blix and Lindberg, 1960). NeuAc, an amino monosaccharide in pyranose form with a molecular weight of 309, is the basic molecule from which other sialic acids are derived by substitution of the amino and hydroxyl groups. NeuAc was first isolated by Gottschalk following interaction of the influenza B virus enzyme with urinary mucoproteins (Gottschalk, 1951).

Sialic acids occur in low concentrations in many living organisms, for instance viruses, bacteria, protozoa, and vertebrates. The plasma level of free sialic acid in healthy adults ranges from 0.5 to 1.2 nmol/ml (Seppala *et al.,* 1990). They are commonly located at the outer cell membrane, where they are linked α-glycosidically in terminal position to galactose, *N*-acetylglucosamine (GlcNAc), *N*-acetylgalactosamine (GalNAc), or other sialic acids. A variety of biological functions has been attributed to the sialic acids (Schauer, 1982). Their strong electronegative charge provides erythrocytes and thrombocytes with a protective layer that prevents aggregation. They facilitate the binding of positively charged molecules and increase the viscosity of glycoproteins in mucous secretions.

Sialic acids act as components of toxin and hormone receptors, including diphtheria, tetanus, cholera, insulin, and thyroid-stimulating hormone (TSH). Ashwell and Morell (1974) first recognized their role as biological masks. Using sialidase they removed the sialic acid residues from radioactively labeled ceruloplasmin exposing the terminal galactose molecules. The resulting asialoceruloplasmin disappeared within minutes from the serum, and increased radioactivity in the liver indicated its degradation (Ashwell and Morell, 1974). Further examples are the rapid elimination of desialylated erythrocytes from the bloodstream and the regression of malignant tumor cells following treatment with sialidiase.

Anabolism of sialic acids

Sialic acids are synthesized from hexose and hexosamine molecules, which are transformed to uridine diphosphate UDP-GlcNAc in the cytoplasm. UDP-GlcNAc is epimerized to *N*-acetylmannosamine (ManNAc) and uridine diphosphate (UDP) by the enzyme UDP-GlcNAc 2-epimerase (Sommar *et al.,* 1972). This is a central step of NANA biosynthesis, which is regulated via feedback inhibition by the end product, cytidine monophosphate *N*-acetylneuraminic acid (CMP-NeuAc). A defect in the feedback mechanism leads to overproduction and lysosomal storage of sialic acids, as seen in sialuria. Similarly, UDP-GlcNAc controls the first enzyme in the pathway leading to its formation, glutamine-fructose-6-phosphate transaminase (Kornfeld *et al.,* 1964). ManNAc is converted to *N*-acetylmannosamine-6-phosphate, *N*-acetylneuraminic acid-9-phosphate, and, finally, NeuAc. In the cell nucleus, *N*-acetylneuraminic acid is activated to CMP-NeuAc with CMP-acylneuraminate synthase and carried to the Golgi apparatus by sialyltransferase (Kolisis and Hervagault, 1986).

Modification of NeuAc

The pathways leading from NeuAc to the other sialic acid derivatives are not fully understood. One of the enzymes involved is *N*-acetylneuraminate mono-oxygenase. This enzyme catalyzes the hydroxylation of NeuAc in the presence of oxygen, iron, ascorbic acid, and NADPH. The product of this reaction is *N*-acetylglucosamine. NeuAc and GlcNAc are acetylated in the positions O-4, O-7, and O-9 by different acetyltransferases (Schauer, 1982). In the Golgi apparatus, several sialyltransferases link sialic acid residues to oligosaccharides, glycoproteins, and glycolipids. The 3 main enzymes found in the smooth endoplasmic reticulum are β-D-galactoside-α-(2→6)-sialyltransferase, β-D-galactoside-α-(2→3)-sialyltransferase, and 2-acetamido-2-deoxy-α-D-galactoside-α-(2→6)-sialyltransferase. The activity of the sialyltransferases is increased by insulin, human chorionic gonadotropin, and platelet-aggregating compounds. Cytidine monophosphate and its derivatives, and infection with Epstein-Barr virus have an inhibiting effect (Schauer, 1985).

Catabolism of sialic acids

The degradation of sialylglycoconjugates takes place in the lysosome. Specific sialidases, also called neuraminidases, release sialic acids from their glycosylic bonds by hydrolysis. Sialidases have been found in many microorganisms and mammals, where they play an important role in the modification of immunological properties of cells. Neuraminidases are also involved in the spreading of viral and bacterial infections (Schauer, 1982). Studies on their substrate specificity revealed that (2→3) glycosidic linkages are split at a higher rate than (2→6) and (2→8) glycosidic bonds. An interesting phenomenon is the relative resistance of 4-O-acetylated sialic acids toward sialidases. Recently, an alternative metabolic route has been detected in horse liver, where the 4-O-acetyl group is removed by an esterase (Schauer, 1985). Unbound sialic acid is released from the lysosome via a proton-driven carrier specific for monosaccharides, which is situated in the lysosomal membrane (Mancini *et al.,* 1989). In the cytoplasm, NANA is separated into 2-acylamido-2-deoxy-D-mannose and pyruvate in a reversible reaction by the enzyme acylneuraminate pyruvate-lyase (NANA-aldolase). The resulting pyruvate supplies the cell with energy and the 2-acylamido-2-deoxy-D-mannose is recycled for the synthesis of sialic acid. Acylneuraminate pyruvate-lyase, therefore, controls the amount of NeuAc available in body tissues (Brunetti *et al.,* 1962).

THE MOLECULAR DEFECT IN SIALIC ACID STORAGE DISEASE

Chromosome 6q

The Finnish population has a characteristic disease heritage due to its relative geographic isolation. More than 30 rare recessive disorders of extreme homogeneity have been identified by positional cloning, and, the gene locus is known in about half of them (Peltonen *et al.,* 1999). In 1992, Haataja and coworkers conducted a genetic linkage analysis of 24 families, where at least one member had SD. They studied 64 gene markers on 19 autosomes by exclusion mapping and concluded that chromosome 2q was a likely candidate site for the disease gene (Haataja *et al.,* 1992). However, extended analysis using 85 restriction fragment length polymorphisms (RFLP) and 86 mi-

crosatellite markers provided evidence that the SD gene mapped to the long arm of chromosome 6. The highest LOD score was obtained for the marker D6S286, where only one recombination event occurred (Haataja *et al.,* 1994b).

To define the SD locus further, Schleutker and colleagues constructed a dense map of 13 microsatellites on a critical chromosomal segment of 10 centiMorgan (cM) length. Linkage disequilibrium mapping enabled them to assign the gene for SD to an 80-kb region between 6q14 and 6q15 (Schleutker *et al.,* 1995a). Three markers, D6S280, D6S406, and D6S456, were found in 75% of parental SD chromosomes (2-5-4 haplotype). The same authors studied the haplotypes of 26 non-Finnish families with SD or ISSD, and compared them with existing data from 50 Finnish SD families. They demonstrated that the two types of sialic acid storage disorders had a common gene locus, but varied in their haplotypes. The majority of SD patients belonged to the 1-3 haplotype, whereas the 7-6 haplotype predominated among infants with ISSD (Schleutker *et al.,* 1995b). Leppanen *et al.* (1996) used the fiber-FISH technique to assemble a detailed genetic map of the chromosomal region between the markers D6S280 and D6S1622, which provided the basis for cloning of the SD gene.

Lysosomal sialic acid transporter

Soon after the first case reports of patients with SASD had been published, it was suggested that the underlying cause for this new inborn error of metabolism was a malfunctioning active transport of NeuAc across the lysosomal membrane (Renlund *et al.,* 1983b). This hypothesis was confirmed in a number of experiments that demonstrated the reduced lysosomal egress of radiolabeled NANA derivatives from SD and ISSD fibroblasts (Mancini *et al.,* 1986; Renlund *et al.,* 1986b; Mendla *et al.,* 1988; Tietze *et al.,* 1989). Mancini and co-workers (1986) developed an elegant technique to study the flux of free sialic acid in and out of the lysosome. They measured the uptake of (^{14}C)NeuAc after purification and resealing of lysosomal membrane vesicles from rat liver. They demonstrated that NeuAc and glucuronic acid (GlcUA) entered the lysosome through a pH-dependent carrier-mediated mechanism (Mancini *et al.,* 1989). Later, the same group extracted the sialic acid carrier with a detergent from the lysosomal membrane and successfully reconstituted it in egg yolk liposomes (Mancini *et al.,* 1992a). Havelaar *et al.* (1998) isolated the functionally active transport protein in several steps with hydroxyapatite, affinity, and ion-exchange chromatography. Substrate specificity testing revealed that the carrier recognized mono- and dicarboxylates in addition to acidic monosaccharides (Havelaar *et al.,* 1998). A similar transport system was found to be present in lysosomal membrane vesicles from healthy humans and patients with SD. The uptake rate for glucuronic acid was high in the control group, intermediate in obligate heterozygotes, and low in patients with SD, which was evidence for a recessive mode of inheritance (Mancini *et al.,* 1991).

SLC17A5 *gene*

Two main cellular transport systems for the exchange of essential metabolic substances and waste products are known to occur in living organisms. The ATP-binding cassette superfamily (ABC) facilitates the active transport of micro- and macromolecules by consumption of ATP, whereas the major facilitator superfamily (MFS) is capable of transporting small molecules only using an osmotic gradient across cellular compartments. ABC and MFS members constitute about 50% of transporters in microorganisms and mammals. The molecular structure of MFS permeases characteristically consists of a single protein with two 6-unit transmembrane domains (Pao *et al.,* 1998). Havelaar and co-authors (1999) tested the kinetic properties of the sialic acid carrier with representative substrates for the 18 MFS subfamilies. The results suggested that it belonged either to the monocarboxylate porter family (MCP, No. 13) or to the anion/cation symporter family (ACS, No. 14), which made them potential candidates for a gene search (Havelaar *et al.,* 1999). Verheijen and colleagues (1999) screened existing cDNA libraries for clones that covered the previously determined critical gene segment on chromosome 6q. They identified a new gene, *SLC17A5*, which encodes a 57-kD polypeptide with a predicted transport function and is mutated in patients with sialic acid storage disorders (Verheijen *et al.,* 1999). One particular mutation, R39C or Salla$_{Fin}$, which changes the amino acid arginine at position 115 to cysteine, is present in 95% of Finnish and Swedish patients with Salla disease. The same mutation was found in a heterozygous form in five non-Scandinavian patients. These patients were classified as intermediate types and showed a more severe clinical picture. In contrast, the children with ISSD had 10 different mutations, including deletions, insertions, missense, and nonsense mutations (Aula *et al.,* 2000). The carrier frequency of Salla$_{Fin}$ is 1.87% in the Salla area and 0.44% in the rest of Finland, indicating a founder effect. A minisequencing test for population-wide genetic screening is available (Syvanen *et al.,* 1992).

Sialin

The gene product expressed by the *SLC17A5* gene consists of 495 amino acids and is homologous to the ACS family, which comprises 40 transport proteins (Verheijen *et al.,* 1999). It was named "sialin" because of its postulated role in the sialic acid storage disorders. This should not be confused with the salivary gland tetrapeptide "sialin" that has a pH-stabilizing effect on human saliva (Coulter *et al.,* 1990). Alignment of the amino acid sequence showed that they lysosomal membrane protein "sialin" is 37% identical and 61% similar to a human brain-specific Na^+-dependent inorganic phosphate cotransporter (hBNPI). This protein is expressed solely in neurons and glia cells, where it is responsible for the import of inorganic phosphate (Ni *et al.,* 1996). Sequence comparison with a sodium-dependent phosphate transport protein (NPT1), which is predominant in human kidneys, indicated 34% identity and 57% similarity.

NPT1 is 467 amino acids long and contributes to the phosphate equilibrium in the proximal renal tubules (Chong *et al.,* 1993). Analysis by computer-aided topology and a hydrophobicity plot suggested that "sialin" has 12 transmembrane regions (TM-domains) and six *N*-glycosylation sites. The fourth TM-domain is characteristic for members of the anion/cation symporter family. Preliminary comparative modeling studies using the SAS/FASTA program revealed partial homology of "sialin" with halorhodopsin and the human glucose transporter type 1 (GTR1).

Halorhodopsin is a light-driven chloride pump found in

Halobacterium salinarum. It functions as a photosynthetic reaction center and consists mainly of membrane-spanning α-helix pairs. Halorhodopsin is homologous to "sialin" from amino acid position 43 to 289 and shows 30% identity.

The human glucose transporter type 1 belongs to the family of glucose transporters and is expressed as integral membrane protein in many human tissues. It is responsible for constitutive or basal glucose uptake and has a broad substrate specificity, which includes various pentoses and hexoses. The glucose transporter is encoded by the *SLC2A1* gene or *GLUT1* gene. *GTR1* contains predominantly α-helices and is homologous to "sialin" between positions 295 and 504, including gap segments. It shows 26.3% identity to "sialin" (Strehle, 2002).

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