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Clinical, biochemical, and molecular diagnosis of a free sialic acid storage disease patient of moderate severity

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Abstract

The allelic autosomal recessive lysosomal storage disorders Salla disease and infantile free sialic acid storage disease (ISSD) result from mutations in *SLC17A5*. This gene codes for sialin, a lysosomal membrane protein that transports the charged sugar, *N*-acetylneuraminic acid (sialic acid), out of lysosomes. ISSD has a severe phenotype with infantile onset, while the Finnish variant, Salla disease, has a milder phenotype with later onset. Both disorders cause developmental delay, and ISSD is generally fatal in early childhood. We describe a 30-month old non-Finnish, Caucasian child with global developmental delay of postnatal onset, language, and motor skills stagnant at a 3–4 month level, hypotonia, and mild but progressive coarsening of facial features. Urinary excretion of free sialic acid was elevated 4.5 times above control. EM of a skin biopsy revealed enlarged secondary lysosomes consistent with oligosaccharide storage. Free sialic acid in fibroblasts was 3.8 ± 0.9 nmol/mg protein (concurrent normal controls, 0.5 ± 0.1); differential centrifugation indicated a lysosomal location. Genomic analysis revealed compound heterozygosity for two new *SLC17A5* mutations. This child's clinical manifestations of a lysosomal free sialic acid storage disease are consistent with her sialin mutations and biochemical findings. The differential diagnosis of postnatal developmental delay should include free sialic acid storage disorders such as ISSD and Salla disease.
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Keywords: Sialic acid; *N*-acetylneuraminic acid; Lysosomal storage; Infantile free sialic acid storage disease; ISSD; Salla disease

Introduction

Lysosomal storage of free sialic acid results from a genetic defect in transport of this charged sugar out of lysosomes [1]. In a mild type of lysosomal free sialic acid storage, i.e., Salla disease common in Finland, newborns develop intellectual impairment gradually. In the more severe allelic variant, i.e., infantile free sialic acid storage disease (ISSD), patients generally die early in childhood or even in utero [2]. Individuals with symptoms of moderate severity are considered to have "intermediate severe Salla disease." There is a correlation between the phenotypes of these three groups of patients and their genetic mutations in *SLC17A5*, the gene that codes for the lysosomal sialic acid transporter, sialin [3–9]. The differential diagnosis of free sialic acid storage also includes sialuria, due to defective feedback inhibition of UDP-GlcNAc-2-epimerase by CMP-sialic acid in the sialic acid synthetic pathway [10]. In contrast to Salla disease and ISSD, the overproduction and accumulation of free sialic acid in sialuria occurs in the cytoplasm of cells rather than in their lysosomes.

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We describe a now 2-year-old girl with clinical, biochemical, histological, and molecular findings typical of a free sialic acid storage disorder. This case emphasizes the need to consider this diagnosis in patients with developmental delay, growth retardation, and mildly coarse facies. Lack of Finnish ethnicity should not preclude investigation of free sialic acid storage, which can be pursued initially by quantitative determination of urinary free sialic acid or thin layer chromatographic screening for oligosaccharides, and later by sialic acid measurements in subcellular fractions of cultured fibroblasts. Molecular studies can confirm the diagnosis.

Methods

SLC17A5 mutation analysis

Mutation analysis was performed as described [11]. In short, genomic DNA was extracted from cultured fibroblasts using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). Total RNA was extracted from cultured fibroblasts using Trizol reagent (Life Technologies, Grand Island, NY). cDNA was synthesized by reverse transcription using 5 μg of total RNA and a SuperScript First-Strand Synthesis System for RT-PCR, according to the manufacturer's protocol (Gibco-BRL, Grand Island, NY). Amplification of the *SLC17A5* gene was performed using 200–400 ng of cDNA, Ready-To-Go PCR Beads (Amersham–Pharmacia Biotech, Piscataway, NJ), and 10 pmol of each primer in a total volume of 25 μL. The PCR conditions were as published [11]. Electrophoresis of the products was performed using 1% agarose. The coding region of sialin, comprising exons 1–11, was amplified using primers previously described [4]. For automated sequencing, we employed a Beckman CEQ 8000 sequencer and CEQ Dye Terminator Cycle Sequencing kit according to the manufacturer's protocol (Beckman–Coulter, Fullerton, CA).

Free sialic acid measurement

After informed consent from the parents, a skin biopsy was obtained from the patient and fibroblasts were cultured as described [7]. Fibroblast content of free sialic acid was assayed by pulsed amperometric detection, as published previously [7]. For subcellular fractionation studies using differential centrifugation, confluent fibroblasts were disrupted in a nitrogen cavitation chamber using 30-psi pressure [8]. The homogenate was centrifuged at different speeds to separate the nuclear, granular (lysosomal), microsomal, and soluble (cytosolic) fractions [8]. Using a previously described assay, β-hexosaminidase activity was measured in each fraction to verify that the lysosomes were not ruptured [7]. Protein was measured using the bicinchoninic acid method [12].

Electron microscopy

Part of the punch skin biopsy, including dermis, was fixed in Trump's fixative, post fixed in 1% osmium tetroxide in sodium cacodylate buffer, stained en bloc with 5% aqueous uranyl acetate and embedded in epon. Thick sections, 1 μm, were cut and stained with toluidine blue. Thin sections were cut at 50–70 nm, stained with uranyl acetate and lead citrate, and examined with Philips EM 201.

Results

Case report

A 9-month-old girl was referred because of developmental delay. She was the first child of unrelated parents, born at full-term by spontaneous vaginal delivery. The four grandparents were of English, German, and Irish origin. A paternal first cousin had autism, but there was no family history of seizures, epilepsy, neuromuscular disorders, or other neurological conditions. Birth weight was 3700 g. Meconium was present at birth, but there were no perinatal complications. The infant was entirely breastfed. Her growth and development were normal until 6 months of age, at which time she was able to roll over. By 9 months, her head control was incomplete, she could not sit, she bore weight only partially and briefly, and she could not babble in strings of syllables but used only open vowel sounds. She had a social smile. The infant could transfer objects, reach out to bat at objects and grasp them, bringing her hands together. She had difficulty feeding with a bottle and dribbled using either a cup or a bottle. Her vision and hearing appeared normal, and she did not vomit frequently. No unusual odors or developmental regression were noted.

On examination at 9 months of age, the girl was of fair complexion (Fig. 1). The length was 72.8 cm (75–90th percentile), weight 7.65 kg (10–25th percentile), and head circumference 44.8 cm (50th percentile). The anterior fontanel was 2 × 2 cm, soft and flat. The nasal bridge was slightly wide, and epicanthal folds were present. The mandible was underdeveloped, as in her mother, but there were no other dysmorphic facial features. She had no neurocutaneous, cardiac, pulmonary, or abdominal abnormalities. Cranial nerves were intact, but she had moderate hypotonia and some shifting tone in her extremities. She had good strength when pushing away or pulling on objects. When pulled to sit, her head lagged. She did not bear weight, and slipped through the examiner's hands. She could not sit independently. Her deep tendon reflexes were 2+, with some increase at the

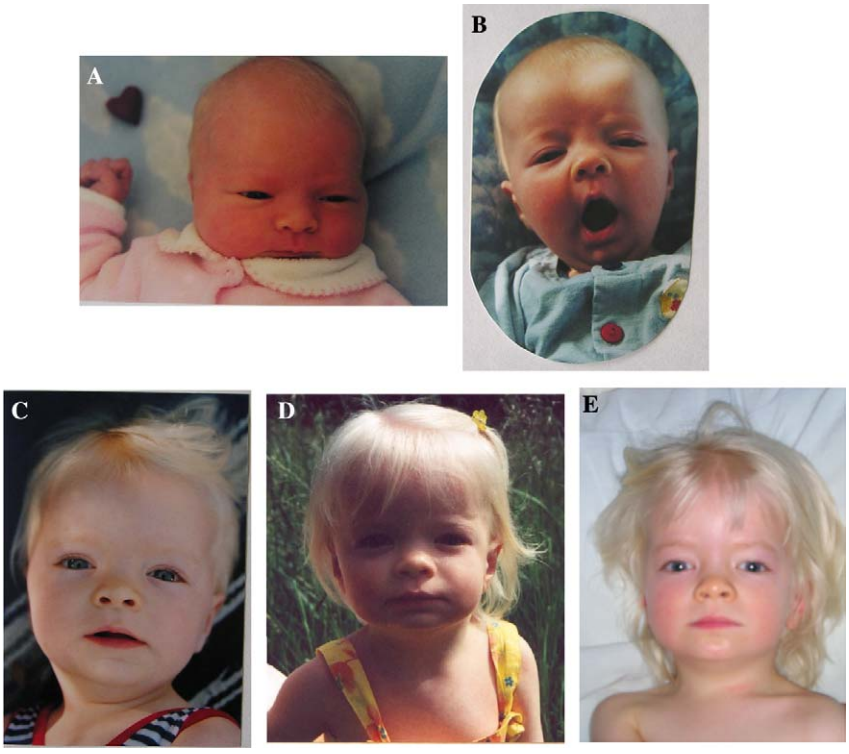

Fig. 1. Facies of the patient. (A) 4 days. (B) 3 months. (C) 11 months. (D) 18 months. (E) 30 months. Note the gradual appearance of coarse facial features.

knees where a bilateral crossed adductor reflex was present. Her toes were tonically upgoing and on stimulation appeared upgoing as well (striatal toes). She had an unusual quality to her movements, reaching out with a fully extended arm to grab objects with a swinging motion. She did not have choreoathetosis or persistent neonatal reflexes. Magnetic resonance imaging at 10 months revealed widespread and profound hypo-myelination throughout the cerebral and cerebellar hemispheres, giving the appearance of a newborn. There was also hypogenesis of the corpus callosum (Fig. 2).

At 12 months, the patient could sit for a few seconds when propped and could bear weight briefly. She was not pulling to stand, but her head control was complete. She lacked a pincer grasp but brought objects to her mouth. She had spells of tonic upward ocular deviation. An EEG showed no epileptiform activity. Her blood pressure was 105/51, pulse 115, height 77 cm (75–90th percentile), weight 8.5 kg (10–25th percentile), and head circumference 46.5 cm (50–75th percentile). Her anterior fontanel measured 3 cm × 3 cm. She had no hepatosplenomegaly. When held in a seated position, her eyes rolled slightly vertically upward and then back down to the midline. Her muscle tone remained poor. Ptosis was present on the left (2 mm) more than the right. Her sensory examination was normal.

At this time, her urinary sialic acid, measured by the thiobarbituric acid assay, showed a non-hydrolyzed value 5.7 times that of the control, while the hydrolyzed value was 4.5 times the control. This indicated an elevation of free sialic acid. Electron microscopic (EM) examination of a skin biopsy showed numerous enlarged lysosomes within keratinocytes, endothelial cells, Schwann's cells, eccrine glands, and myocytes (Fig. 3). Karyotype, lysosomal enzymes (aryl sulfatase A, β-galactosidase, α-L-fucosidase, and galactocerebrosidase), peroxisomal profile (urine pipecolic acid and plasmalogens), urinary organic acids, urinary and CSF amino acids, biotinidase, carnitines, transferrin isoforms, CBC, cholesterol, folate, electrolytes, lactate, and ammonia were all normal. An audiology evaluation was normal and ophthalmologic examination revealed mild optic pallor. Electrophysiological studies were abnormal and supported a diagnosis of peripheral neuropathy. The tibial motor nerve conduction appeared to be normal but the F-wave was highly prolonged. Sural nerve action potential was normal with 10 mV. Peroneal nerve conduction was low and there was no F-wave apparent.

At 26 months of age, the patient's development had progressed slightly. She was not sitting independently, but she did bear weight occasionally. Her overall health was good, although it took at least an hour to feed her a meal, and vomiting was frequent. Her vision was 20/80. She tracked well and had normal hearing. The blood pressure was 108/70, pulse 123, height 86.5 cm (25–50th percentile), weight 10.25 kg (< 3rd percentile), and head circumference 48 cm (25th percentile). There was mild coarsening of her facial features, but no

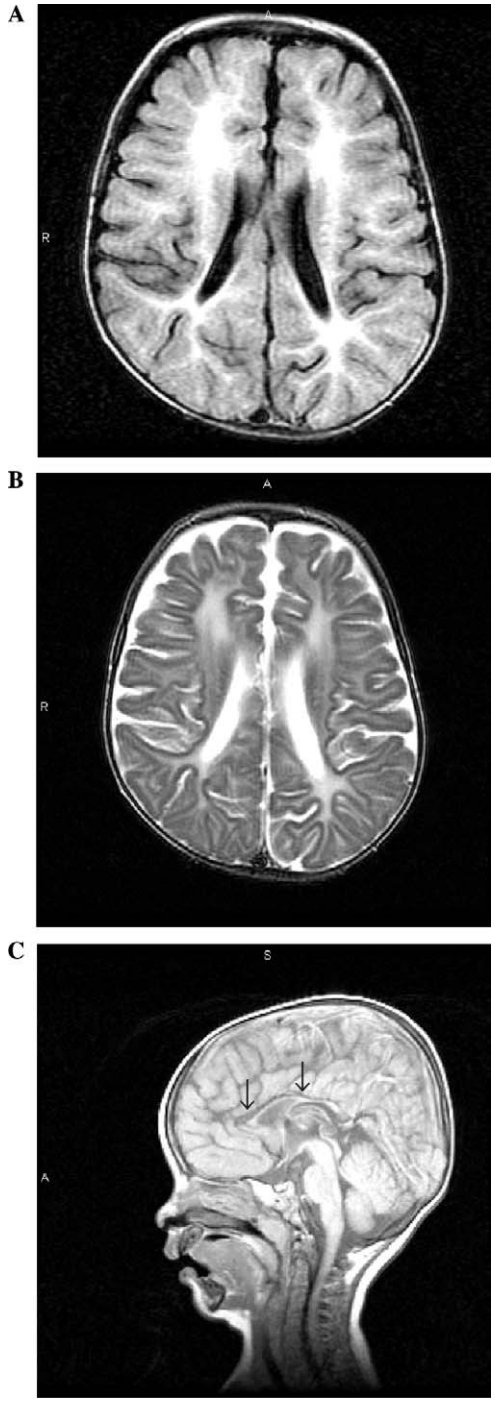

Fig. 2. Brain NMR of the patient at 10 months of age. (A) Axial T1-weighted. (B) Axial T2-weighted. (C) Sagittal midline T1-weighted. Note significant hypomyelination and small corpus callosum (arrows).

hepatosplenomegaly, cardiac or pulmonary abnormalities, or joint contractures. She had frequent upward eye rolling episodes, i.e., oculogyric spasms, some associated with vomiting. She continued to show moderate to severe hypotonia with a significant head lag and an inability to sit unsupported. Strength was normal, and the rest of her neurological examination was unchanged.

Fibroblast sialic acid measurements

The free sialic acid levels in our patient's cultured fibroblasts were, on average, 8-fold increased over concurrently measured controls, with repeated levels of 2.67, 3.55, 3.90, and 4.86 nmol/mg protein (Table 1). Subcellular fractionation studies showed that the distribution of free sialic acid in our patient's fibroblasts correlated directly with that of the lysosomal enzyme hexosaminidase (data not shown), indicating a lysosomal location for the sialic acid. Most of the free sialic acid (72%) was within the granular compartment and very little was present in the soluble fraction (Table 2), consistent with the distribution of free sialic acid found in fibroblasts of patients with lysosomal free sialic acid storage disease. In normal fibroblasts, approximately half of the intracellular free sialic acid resides in the cytosolic compartment, and in sialuria cells the vast majority of the free sialic acid is also cytosolic.

SLC17A5 mutation analysis

The *SLC17A5* gene was analyzed using both cDNA and genomic DNA. The patient was compound heterozygous for one new splice site mutation and one new missense mutation (Fig. 4). A c291G > A change in the last base of exon 2 caused a splicing defect, deletion of the 197 bases of exon 2 (data not shown), and a frameshift leading to a premature stop codon after 32 amino acids. The patient's second mutation was a G > A mutation at position c1226 in exon 9, leading to alteration of a highly conserved amino acid within a transmembrane region, i.e., G409E. Each mutation was present in one parent.

The patient also showed two heterozygous polymorphisms, i.e., c85G > A leading to A29T in exon 1 on the paternal allele and c948G > A leading to M316I in exon 7. Both polymorphisms change non-conserved amino acids within similar amino acids groups. The A29T polymorphism is known as single nucleotide polymorphism dbSNP rs545973.

Discussion

Sialic acid, or *N*-acetylneuraminic acid, participates in several critical biological processes as the terminal, charged sugar on N-linked glycoproteins and glycolipidsfunctions on the surface of cells to assist in interactions with other cells. Within the lysosome, free sialic acid forms from the action of the acid hydrolase neuraminidase, or sialidase, and is removed from the lysosome by the integral lysosomal membrane transporter called sialin. In lysosomal disorders of free sialic acid, this sugar accumulates in lysosomes along with glucuronic acid, which is also a ligand for sialin [13]. How the

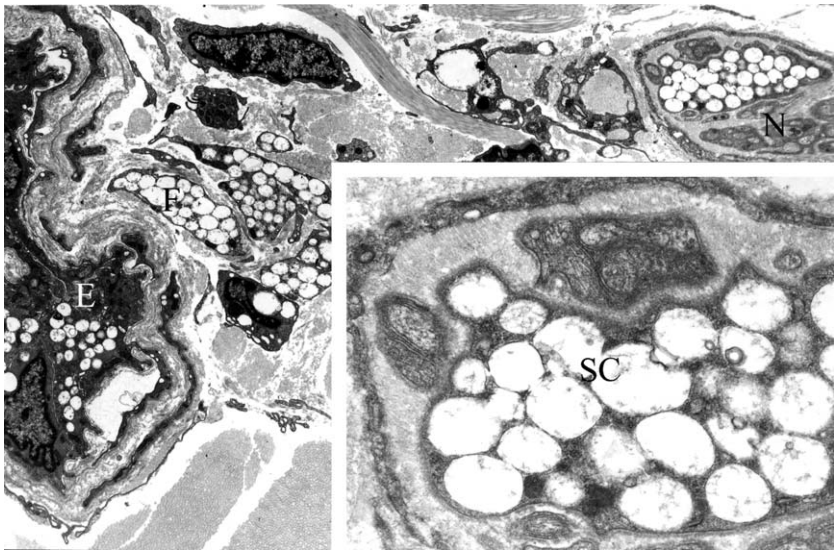

Fig. 3. Electron micrograph of a skin biopsy. Dermis revealing blood vessels with endothelial cells (E) and pericytes, a nerve (N) bundle with Schwann's cells (SC), and fibroblasts (F). The endothelial cells, fibroblasts, and Schwann's cells have numerous enlarged secondary lysosomes (3860×). Inset: Schwann's cell containing enlarged secondary lysosomes, most of which are electron lucent; some contain fine fibrillar material (17,550×).

Table 1
Free sialic acid levels in patient fibroblasts

	Free sialic acid (nmol/mg protein)	
	N	Mean ± SD
Patient	4[a]	3.8 ± 0.9
Concurrent controls	4[b]	0.5 ± 0.1
Published values[c]		
Controls	11[b]	1.0 ± 0.6
Salla disease	6[b]	10.0 ± 2.9
ISSD	5[b]	139 ± 92

[a] *N*, number of determinations.
[b] *N*, number of different patients.
[c] From Seppala et al. [12].

elevation of lysosomal free sialic acid contributes to the pathophysiology and the clinical picture of affected patients is not fully understood and remains under investigation.

The lysosomal free sialic acid storage disorders, which are inherited in an autosomal recessive fashion, have a broad spectrum of clinical presentations. Salla disease represents the mildest type and occurs predominantly in Finland, with a few cases in Sweden, Denmark, and recently North America [4,14–18]. Salla disease was named after the Lapland community of northern Finland, where most of the grandparents of affected individuals were born. Indeed, Salla disease patients all display the Finnish founder mutation, R39C, in *SLC17A5*. Urinary and fibroblast free sialic acid levels are elevated approximately 10-fold in Salla disease [1].

ISSD represents the most severe type of lysosomal free sialic acid storage disease, sometimes manifesting with hydrops fetalis and generally resulting in early death. ISSD has no geographic predominance. Most patients have been reported in Europe, but we recently described the genetic and clinical findings of a North American ISSD patient [11]. A large number of different *SLC17A5* mutations have been found in ISSD patients. Urinary and fibroblast free sialic acid are elevated approximately 100-fold in ISSD [1].

There also exists a type of lysosomal free sialic acid storage that is intermediate in severity between Salla disease and ISSD, and this variant describes our patient. She exhibited a clinical course less severe than typically seen in ISSD, but with more severe developmental problems than usually observed in Salla disease. Her brain MRI showed hypomyelination and a small corpus callosum, similar to a case reported in a 2-year-old with molecularly proven intermediate severe Salla disease [19]. Most of the sialic acid in our patient's cultured fibroblasts was located within the cells' lysosomal fraction (Table 2), and the diagnosis was confirmed by finding compound heterozygous mutations in *SLC17A5*. One confounding finding was that our patient's urine and fibroblast free sialic acid levels were increased less than the 10-fold elevation usually seen in Salla disease. This curious, reproducible finding has several possible implications. First, it suggests that lysosomal free sialic acid levels can be influenced by unknown factors, such as the rate of synthesis of glycoconjugates or the level of neuraminidase activity. Second, it expands the range of sialic acid levels that can be associated with moderately severe lysosomal free sialic acid storage. Finally, it raises the possibility that one of the patient's two *SLC17A5* mutations allows significant residual sialin function.

This case illustrates the difficulty inherent in diagnosing a free sialic acid storage disease, and the importance of considering this diagnosis in the face of progressive

Table 2
Subcellular fractionation of free sialic acid in fibroblasts

Subcellular Fraction	Free sialic acid recovered (% of total)				
	Normal controls		Sialuria[a]	ISSD[b]	Patient[c]
	Published[a]	Concurrent			
Nuclear	18	12	5	6	18
Granular[d]	21	34	4	77	72
Microsomal	6	5	2	8	8
Soluble	54	49	89	10	2

[a] Seppala et al. [12].
[b] Tietze et al. [8].
[c] Mean of duplicate determinations.
[d] Contains lysosomes, mitochondria, and other membrane-limited vesicles.

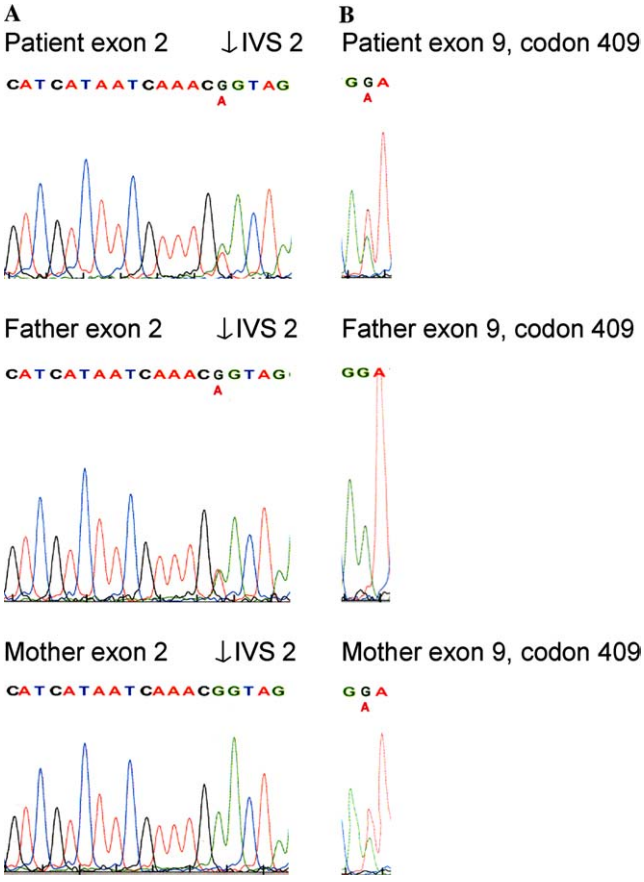

Fig. 4. Sequencing results of *SLC17A5* in the patient. (A) Sequencing results of the splice site of exon 2 of the genomic DNA of the patient and her parents. A heterozygous G > A mutation exists in the last base of exon 2 of the patient and her father (arrow). The mother's sequence is normal. (B) Sequencing results of exon 9 of the patient and her parents. Codon 409 contains a heterozygous G > A missense mutation in the patient and her mother (arrow). The father's sequence is normal.

developmental delay of postnatal onset. A variety of tests were performed before the findings of enlarged lysosomes in the skin biopsy and an elevated excretion of free sialic acid led to the diagnosis. The skin biopsy pointed to a lysosomal storage disease, specifically one involving the storage of small molecules like sialic acid or an oligosaccharide [20]. The morphological findings in our patient were identical to those observed in skin and in conjunctiva of sialic acid storage disease patients [6,21]. However, a skin biopsy does not always show abnormal findings in lysosomal storage diseases, including those involving free sialic acid [18]. Only a high index of suspicion led to measurement of urinary free sialic acid. Obtaining urine oligosaccharides can be a useful screening tool for these disorders if a laboratory performs both thiobarbituric acid (TBA) and thin-layer chromatography (TLC) based oligosaccharide screening and is proficient in the detection of free sialic acid.

Interestingly, our patient at the age of 12 months showed electrophysiological signs of peripheral dysmyelination, previously reported in Salla disease patients [22]. The dysmyelination might contribute to the patient's clinical phenotype.

We believe that free sialic acid storage disorders are underdiagnosed because of their non-specific clinical findings, the difficulty in obtaining reliable free sialic acid determinations, and physicians' lack of awareness of the existence of this disease entity [18,19,23]. Lysosomal free sialic acid storage disorders should be included in the differential diagnosis of developmental delay of postnatal onset, of white matter disease with hypomyelination, and of lysosomal storage disorders proven by the demonstration of enlarged lysosomes.

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References

[1] P. Aula, W.A. Gahl, Disorders of free sialic acid storage, in: C.R. Scriver, A.L. Beaudet, D. Valle, W.S. Sly (Eds.), The Metabolic

and Molecular Bases of Inherited Disease, eighth ed., McGraw-Hill, New York, 2001, pp. 5109–5120.
[2] E. Lemyre, P. Russo, S.B. MelanSon, R. Gagné, M. Potier, M. Lambert, Clinical spectrum of infantile free sialic acid storage disease, Am. J. Med. Genet. 82 (1999) 385–391.
[3] J. Schleutker, P. Leppänen, J.E. Månsson, A. Erikson, J. Weissenbach, L. Peltonen, P. Aula, Lysosomal free sialic acid storage disorders with different phenotypic presentations—infantile-form sialic acid storage disease and Salla disease—represent allelic disorders on 6q14-15, Am. J. Hum. Genet. 57 (1995) 893–901.
[4] F.W. Verheijen, E. Verbeek, N. Aula, C.E. Beerens, A.C. Havelaar, M. Joosse, L. Peltonen, P. Aula, H. Galjaard, P.J. van der Spek, G.M. Mancini, A new gene, encoding an anion transporter, is mutated in sialic acid storage diseases, Nat. Genet. 23 (1999) 462–465.
[5] P. Aula, S. Autio, K.O. Raivio, J. Rapola, C.J. Thodén, S.L. Koskela, I. Yamashina, Salla disease: a new lysosomal storage disorder, Arch. Neurol. 36 (1979) 88–94.
[6] M. Renlund, P. Aula, K.O. Raivio, S. Autio, K. Sainio, J. Rapola, S.L. Koskela, Salla disease: a new lysosomal storage disorder with disturbed sialic acid metabolism, Neurology 33 (1983) 57–66.
[7] M. Renlund, F. Tietze, W.A. Gahl, Defective sialic acid egress from isolated fibroblast lysosomes of patients with Salla disease, Science 232 (1986) 759–762.
[8] F. Tietze, R. Seppala, M. Renlund, J.J. Hopwood, G.S. Harper, G.H. Thomas, W.A. Gahl, Defective lysosomal egress of free sialic acid (*N*-acetylneuraminic acid) in fibroblasts of patients with infantile free sialic acid storage disease, J. Biol. Chem. 264 (1989) 15316–15322.
[9] N. Aula, P. Salomäki, R. Timonen, F. Verheijen, G. Mancini, J.E. Månsson, P. Aula, L. Peltonen, The spectrum of SLC17A5-gene mutations resulting in free sialic acid-storage diseases indicates some genotype–phenotype correlation, Am. J. Hum. Genet. 67 (2000) 832–840.
[10] J.G. Leroy, R. Seppala, M. Huizing, G. Dacremont, H. De Simpel, R.N. Van Coster, E. Orvisky, D.M. Krasnewich, W.A. Gahl, Dominant inheritance of sialuria, an inborn error of feedback inhibition, Am. J. Hum. Genet. 68 (2001) 1419–1427.
[11] R. Kleta, D.J. Aughton, M.J. Rivkin, M. Huizing, E. Strovel, Y. Anikster, E. Orvisky, M. Natowicz, D. Krasnewich, W.A. Gahl, Biochemical and molecular analyses of infantile free sialic acid storage disease in North American children, Am. J. Med. Genet. A 120 (2003) 28–33.
[12] R. Seppala, F. Tietze, D. Krasnewich, P. Weiss, G. Ashwell, G. Barsh, G.H. Thomas, S. Packman, W.A. Gahl, Sialic acid metabolism in sialuria fibroblasts, J. Biol. Chem. 266 (1991) 7456–7461.
[13] H.J. Blom, H.C. Andersson, R. Seppala, F. Tietze, W.A. Gahl, Defective glucuronic acid transport from lysosomes of infantile free sialic acid storage disease fibroblasts, Biochem. J. 268 (1990) 621–625.
[14] R. Norio, The Finnish disease heritage III: the individual diseases, Hum. Genet. 112 (2003) 470–526.
[15] T.T. Varho, L.E. Alajoki, K.M. Posti, T.T. Korhonen, M.G. Renlund, S.R. Nyman, M.L. Sillanpaa, P.P. Aula, Phenotypic spectrum of Salla disease, a free sialic acid storage disorder, Pediatr. Neurol. 26 (2002) 267–273.
[16] A. Erikson, N. Aula, P. Aula, J.E. Mansson, Free sialic acid storage (Salla) disease in Sweden, Acta Paediatr. 91 (2002) 1324–1327.
[17] P. Sonderby Christensen, P.H. Kaad, J.R. Ostergaard, Two cases of Salla disease in Danish children, Acta Paediatr. 92 (2003) 1357–1358.
[18] R.A. Martin, R. Slaugh, M. Natowicz, K. Pearlman, E. Orvisky, D. Krasnewich, R. Kleta, M. Huizing, W.A. Gahl, Sialic acid storage disease of the Salla phenotype in American monozygous twin female sibs, Am. J. Med. Genet. A 120 (2003) 23–27.
[19] T. Linnankivi, T. Lonnqvist, T. Autti, A case of Salla disease with involvement of the cerebellar white matter, Neuroradiology 45 (2003) 107–109.
[20] A. Prasad, E.M. Kaye, J. Alroy, Electron microscopic examination of skin biopsy as a cost-effective tool in the diagnosis of lysosomal storage diseases, J. Child Neurol. 11 (1996) 301–308.
[21] M. Tondeur, J. Libert, E. Vamos, F. Van Hoof, G.H. Thomas, G. Strecker, Infantile form of sialic acid storage disorder: clinical, ultrastructural, and biochemical studies in two siblings, Eur. J. Pediatr. 139 (1982) 142–147.
[22] T. Varho, S. Jaaskelainen, U. Tolonen, P. Sonninen, L. Vainionpaa, P. Aula, M. Sillanpaa, Central and peripheral nervous system dysfunction in the clinical variation of Salla disease, Neurology 55 (2000) 99–104.
[23] D.A. Wenger, S. Coppola, S.L. Liu, Lysosomal storage disorders: diagnostic dilemmas and prospects for therapy, Genet. Med. 4 (2002) 412–419.