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Free Sialic Acid Storage Disease without sialuria

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Abstract

We performed high resolution in vitro proton nuclear magnetic resonance spectroscopy on CSF and urine samples of 44 patients with leukodystrophies of unknown cause. Free sialic acid was elevated in CSF of two siblings with mental retardation and mild hypomyelination. By contrast, urinary excretion of free sialic acid in urine was normal on repeated testing by two independent methods. Both patients were homozygous for the K136E mutation in *SLC17A5*, the gene responsible for the free sialic acid storage diseases. Our findings demonstrate that mutations in the *SLC17A5* gene have to be considered in patients with hypomyelination, even in the absence of sialuria.

Keywords

Free Sialic Acid Storage Diseases; Leukodystrophy; NMR Spectroscopy

Introduction

Mutations in the *SLC17A5* gene encoding a lysosomal transporter called sialin are associated with the free sialic acid storage diseases – Salla disease (or the Finnish type of sialuria) and the more severe infantile free sialic acid storage disease (ISSD)[1]. Both are characterized by the abnormal retention of free sialic acid into the lysosome (OMIM 604369 and OMIM 269920) and are termed SASD. SASD patients usually present during the first year of life with hypotonia, ataxia and nystagmus. Cerebellar ataxia remains a prominent feature as disease progresses, further associated with spasticity and severe psychomotor delay. These symptoms are usually well correlated with the brain MRI pattern which is mostly characterized by diffuse supratentorial hypomyelination, thin corpus callosum and cortical and cerebellar atrophy[2]. So far, the elevation of free sialic acid in urine (5 to 20 times normal) has been considered the biochemical hallmark of the disease, and was

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observed as early as 3 days of age[3]. Patients' cultured skin fibroblasts also display elevated free sialic acid[4], while enlarged lysosomal vacuoles are often seen in lymphocytes and fibroblasts. The vast majority of patients – up to 91% – diagnosed so far with Salla disease harbor a homozygous R39C missense *SLC17A5* mutation[5]. Here we describe two siblings with SASD identified by the sole presence of elevated free sialic acid in the CSF but without sialuria on repeated testing.

Subjects and Methods

Forty-four patients with leukodystrophies of unknown cause and a hypomyelinating or demyelinating pattern on brain MRI were recruited at the National Institutes of Health by participating in a research protocol on the leukodystrophies that was approved by the Institutional Review Board of the National Institute of Neurological Disorders and Stroke. The patients or their legal guardians gave their written informed consent and assent including permission for the nerve biopsies.

A wide panel of metabolic and genetic investigations was performed in all patients and showed no abnormality (Supplementary methods). In order to identify new metabolic abnormalities, frozen urine and CSF (stored at −80°C) were prepared for 1H-NMR spectroscopy (H-NMRS) with minimal handling (Supplementary methods).

After significant findings by H-NMRS in the CSF of two patients, further investigations of free sialic acid metabolism were conducted. In addition to HNMRS, urinary sialic acid levels were determined by quantitative colorimetric assay[6] and compared to urine samples from age-matched controls[7]. Sialic acid was also measured in fibroblasts as described[8]. All exons of the *SLC17A5* gene (GenBank NM_012434) were amplified from the patient's genomic DNA by use of specific intronic primers (primer sequences and conditions used in the sequencing analysis are available upon request).

Results

Among the cohort of 44 patients with leukodystrophies of unknown etiology, high resolution in vitro H-NMR spectroscopy of CSF revealed an increased concentration of free sialic acid in two siblings (Figure 1). Free sialic acid was 28 and 29 μmol/l respectively in the patients' CSF that consisted of a 3-fold elevation compared to a large cohort of 250 patients with various neurological conditions (8.9 ± 4.1μmol/l, unpublished data). These values were comparable to the levels of free sialic acid measured by H-NMRS in the CSF of other patients with Salla disease (R.A Wevers, unpublished data). By contrast, the levels of free sialic acid in random urine samples measured by the thiobarbituric acid assay were normal compared to age-matched controls (Table 1). These normal findings were confirmed by a 24h urine collection in both patients performed 3 years after their initial visit (Table 1). The levels of urinary free sialic acid were assessed independently by H-NMRS, which confirmed the normal urinary excretion of free sialic acid in our patients (data not shown). Free sialic acid in fibroblasts was mildly elevated in patient 1–3.1 nmol/mg protein, as well as in patient 2–2.6 nmol/mg protein (control range, 0.2–1.8 nmol/mg protein).

Due to the elevation of free sialic acid in the CSF of both patients, the diagnosis of SASD was suspected. *SLC17A5* sequencing revealed an A>G homozygous transition at cDNA position 406 (exon 3), inherited from both parents, which predicted Lys136Glu change (p.K136E) (Supplementary figure 1).

The patients were two siblings originating in the Dominican Republic, with no known consanguinity. They were born after normal pregnancy and delivery. The main neurological features of both patients are described in Table 2 and compared to previously reported

patients with the same homozygous mutation.[9] Apart from general hypotonia, neurological examination was unremarkable in patient 1 and revealed only a mild dysmetria with a slight wide-based gait in patient 2.

Brain MRI in both siblings showed diffuse but mild symmetric T2-weighted signal hyperintensity throughout the supratentorial white matter with mostly isointense white matter on T1 sequences (Supplementary figure 2). The brainstem and cerebellum exhibited a normal myelination pattern. This hypomyelination pattern was associated with a thin corpus callosum but no cortical or cerebellar atrophy (Supplementary figure 2).

Electromyography, nerve conduction velocities, somatosensory and brainstem evoked potentials were normal. Considering the possibility of peripheral nerve involvement in hypomyelinating leukodystrophies[10, 11], a sural nerve biopsy was performed in both patients and showed normal light and electron microscopic examination, but fatty vacuolar inclusions in the cytoplasm of the Schwann cells were present (Supplementary figure 3).

Discussion

We describe two siblings with a homozygous K136E mutation in the *SLC17A5* gene presenting essentially with mental retardation in the context of mild hypomyelination. This is, to our knowledge, the first report of *SLC17A5* mutations associated with normal urinary excretion of free sialic acid, which we assessed by two independent methods. The phenotype of our patients best fits the Salla disease form of SASD. In contrast to the patient reported by Biancheri et al, and to Salla patients harbouring the classical R39C mutation[9], our patients presented with mental retardation but almost absence of motor impairment.

SASD have been defined by the constant presence of free sialic acid in urine[3]. Considering that molecular investigations of the *SLC17A5* gene have always been based upon the presence of sialuria in patients, the incidence of SASD disease is likely to have been underestimated. The levels of free sialic acid in urine of Salla patients are quite variable (5 to 20 fold) and are probably influenced by unknown mechanisms including the manner by which the kidney handles free sialic acid. Urinary free sialic acid is also known to decrease with age[7]. Accordingly, we confirmed that the levels of free sialic acid excreted by our patients were comparable to age matched controls. Overall, the mechanisms of the excessive urinary excretion of the generally intra-lysosomal free sialic acid have never been established. It was recently suggested that, in addition to sialic acid, sialin can also act as a transporter for aspartate[12]. However, aspartate levels have never been reported to be elevated in the urine of SASD patients. Therefore, the importance of our findings lies in the fact that the diagnosis of SASD is susceptible to be overlooked in a substantial number of patients, including adults.

In view of the unusual phenotype and the absence of sialuria, the investigation of the *SCL17A5* gene was driven by the elevation of free sialic acid in both patients' CSF by H-NMR spectroscopy. The absence of derivation or extraction procedure for NMRS analyses allows the detection and quantification of a wide variety of metabolites of a different nature. Our study further illustrates the contribution of NMRS of body fluids to the discovery and the characterization of several inborn errors of metabolism[13–16]. Although elevation of free sialic acid in cultured skin fibroblasts has thus far been present in all SASD patients, this test is suitable for diagnostic confirmation rather than for screening purposes.

The K136E mutation was first reported in a heterozygous state in association with the classical R39C mutation in an SASD patient presenting with a severe form of the disease[5]. A homozygous K136E mutation was subsequently identified in another patient with a severe clinical picture combining an early onset of global motor and cognitive delay with

hypomyelination of both the supra- and infratentorial white matter[9]. The severity of the clinical and MRI presentation was attributed to the mislocalization of the sialin transporter associated with the K136E mutation[17]. Further studies have challenged this hypothesis by reporting normal intracellular trafficking of the sialin transporter in cell lines harbouring K136E homozygous mutation obtained by mutagenesis[18]. The relatively mild phenotype of our patients further challenges the genotype-phenotype correlation previously suggested for this rare mutation. Polymorphisms in the *SLC17A5* gene or other genes involved in the metabolism of free sialic acid may account for this phenotype discrepancy. Due to the altered free sialic acid intracellular metabolism, sialylation of some key components of the myelin may also be affected, which could vary significantly from patient to patient regardless of the mutation.

In conclusion, mutations in the *SLC17A5* gene have to be considered in patients with hypomyelination even in the absence of increased urinary free sialic acid. Our findings also extend the phenotype associated with mutations in the *SLC17A5* gene. Measuring free sialic acid in CSF by H-NMRS is a useful assay to screen for SASD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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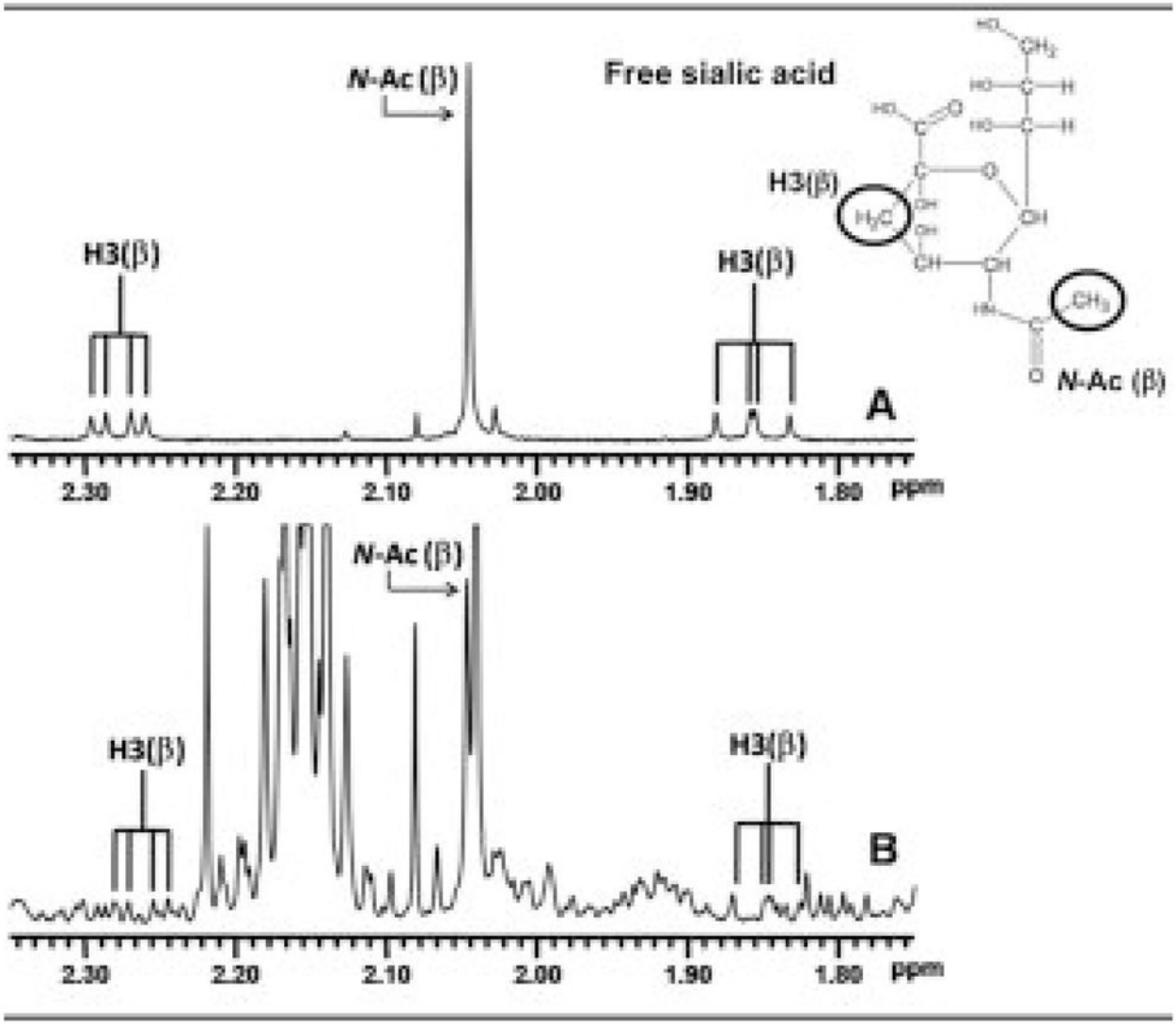

Figure 1.

Identification of free sialic acid by ^{1}H-nuclear magnetic resonance spectroscopy (H-NMRS). (A) Model compound of free sialic acid run at pH 2.5. (B) Cerebrospinal fluid (CSF) sample from the patients homozygous for the K136E mutation in the SLC17A5 gene. Free sialic acid shows as a prominent singlet resonance at 2.05 ppm and other resonances at 1.85 and 2.26 ppm. Note that, except for the first 4 to 6 months of life when greater values can be observed, free sialic acid CSF level is independent of age (data not shown). N-Ac = *N*-acetyl protons of the beta anomer of free sialic acid; H3 = resonances from protons attached to carbon at position 3 of the beta anomer of free sialic acid.

Table 1

Values of Urine Free Sialic Acid in Patients with the Salla Type of Sialic Acid Storage Disease and Age-Matched Control Subjects Measured as Described Previously,[6] Compared with the Previously Reported Patient with the Same Homozygous K136E Mutation[9]

	Patient 1		Patient 2		Biancheri and Colleagues'[9] Patient
	Random Sample	24-Hour Urine Collection	Random Sample	24-Hour Urine Collection	Random Sample
Age, yr	17	19.5	9	12	3
Urine free sialic acid concentration (range), μmol/mmol creatinine	32 (8–35)[a]	31 (8–35)[a]	43 (11–46)[b]	34 (11–46)[b]	140 (18–62)

[a] Values for age-matched control subjects (17–20 years old; n = 19).

[b] Values for age-matched control subjects (8.5–12.5 years old, n = 15).

Table 2

Clinical Characteristics of Two Siblings Homozygous for the K136E Mutation in the SLC17A5 Gene and Comparison with a Previously Reported Patient[9]

Characteristics	Patient 1	Patient 2	Biancheri and Colleagues'[9] Patient
Age of onset, mo	5	2	1st
Gaze-evoked nystagmus	+ (transient)	+ (transient)	+
Generalized hypotonia	+	+	+
Truncal cerebellar ataxia	−	−	+
Psychomotor development			
Sitting, mo	8	5	30
Walking, mo	18	28	Not acquired
Best speech	2-word sentences	2-word sentences	Few single words
Best cognitive ability	4-year equivalent	4-year equivalent	15m equivalent
Age at examination, yr	19.5	12	3
Nystagmus	−	−	+
Dysmetria UL	−	+	++
Gait ataxia	−	Wide based gait	NA
Reflexes LL	Normal	Normal	Brisk
Plantar responses	Flexor	Flexor	Extensor
Spasticity	−	−	ND

Minus sign = absence; UL = upper limbs; NA = not appropriate; LL = lower limbs; ND = not determined.