14. Post MJ, Yiannoutsos C, Simpson D, et al. Progressive multifocal leukoencephalopathy in AIDS: are there any MR findings useful to patient management and predictive of patient survival? AIDS Clinical Trials Group, 243 Team. AJNR Am J Neuroradiol 1999;20:1896–1906.
15. Shintaku M, Matsumoto R, Sawa H, Nagashima K. Infection with JC virus and possible dysplastic ganglion-like transformation of the cerebral cortical neurons in a case of progressive multifocal leukoencephalopathy. J Neuropathol Exp Neurol 2000;59:921–929.
16. Del Valle L, Delbue S, Gordon J, et al. Expression of JC virus T-antigen in a patient with MS and glioblastoma multiforme. Neurology 2002;58:895–900.
17. Raj GV, Khalili K. Transcriptional regulation: lessons from the human neurotropic polyomavirus, JCV. Virology 1995;213:283–291.
18. Messam CA, Hou J, Gronostajski RM, Major EO. Lineage pathway of human brain progenitor cells identified by JC virus susceptibility. Ann Neurol 2003;53:636–646.
19. Lima MA, Drislane FW, Koralnik IJ. Seizures and their outcome in progressive multifocal leukoencephalopathy. Neurology 2006;66:262–264.

De Novo *STXBP1* Mutations in Mental Retardation and Nonsyndromic Epilepsy

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We sequenced genes coding for components of the SNARE complex (*STX1A, VAMP2, SNAP25*) and their regulatory proteins (STXBP1/Munc18-1, SYT1), which are essential for neurotransmission, in 95 patients with idiopathic mental retardation. We identified de novo mutations in *STXBP1* (nonsense, p.R388X; splicing, c.169+1G>A) in two patients with severe mental retardation and nonsyndromic epilepsy. Reverse transcriptase polymerase chain reaction and sequencing showed that the splicing mutation creates a stop codon downstream of exon-3. No de novo or deleterious mutations in *STXBP1* were found in 190 control subjects, or in 142 autistic patients. These results suggest that *STXBP1* disruption is associated with autosomal dominant mental retardation and nonsyndromic epilepsy.

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Mental retardation (MR), the most frequent severe handicap of children, is defined by the presence of significant limitations in cognitive and adaptive behavior.[1] Most MR genes identified so far are either located on the X chromosome or are associated with an autosomal recessive mode of inheritance. Only a small number of autosomal dominant MR genes are known, in part, because MR results in lower reproductive fitness, decreasing the likelihood of identifying families that are amenable to linkage analysis. The fact that de novo copy number variants represent the most commonly recognized cause of MR indicates that monoallelic lesions are sufficient to cause MR and raises the possibility that smaller de novo genetic lesions, such as point mutations, also represent a major cause of MR. The identification of de novo point mutations, however, must rely on the sequencing of candidate genes.

Defects in synaptic function are thought to underlie many common neurodevelopmental brain disorders, including autism and MR.[1,2] One of the key events of interneuronal communication at the synapse is the release of neurotransmitters by Ca^{2+}-triggered synaptic vesicle exocytosis. This vesicular fusion event is mainly mediated by a well-characterized group of SNARE proteins comprising the synaptic vesicle protein synaptobrevin-2/VAMP2, and plasma membrane proteins syntaxin-1A/STX1A and SNAP25, which altogether bring and fuse synaptic vesicles at the presynaptic plasma membrane, releasing their neurotransmitter content into the synaptic cleft.[3,4] This process is regulated by Munc18-1/STXBP1, which is essential for SNARE complex formation and synaptic vesicle fusion, and by synaptotagmin-1/SYT1, the main Ca^{2+} sensor in neurons that mediates fast synaptic vesicle fusion after Ca^{2+} influx.[3,5–8]

We postulated that de novo mutations in autosomal genes that are involved in neurotransmission could lead to cognitive deficits in humans. Specifically, we sequenced the coding exons and their flanking intronic regions of genes encoding the main components of the synaptic SNARE machinery *(STX1A, VAMP2, SNAP25),* together with those of their regulatory proteins (*STXBP1* and *SYT1*), in 95 patients with idiopathic MR. We report the identification of de novo truncating and splicing mutations in *STXBP1* in two patients with MR and nonsyndromic epilepsy.

Subjects and Methods

Subjects and DNA Screening

Ninety-five French Canadian sporadic cases of idiopathic MR without growth abnormalities or specific dysmorphic features were recruited for this study. A subset of the MR patients (25/95) also displayed some forms of epilepsy. Moreover, cohorts of 142 patients with autism spectrum disorder (ASD) and 190 healthy ethnically matched individuals were studied. Additional details concerning the MR and ASD cohorts are provided in the supplementary materials. Blood samples were collected from all members of these cohorts, as well as from their parents after approval by institutional ethics committees. Genomic DNA was extracted from blood samples using the Puregene DNA kit (Quigan, Mississauga, ON, Canada). Paternity and maternity of each individual of all families were confirmed using six informative unlinked microsatellite markers (D2S1327, D3S1043, D4S3351, D6S1043, D8S1179, D10S677). Polymerase chain reaction (PCR) amplification of the exons and intronic splice junctions of *STX1A, VAMP2, SNAP25, STXBP1* (isoforms a,b), and *SYT1,* and subsequent sequencing and mutation analyses were conducted according to standard procedures (see supplementary materials).

RNA Analysis

Total cellular RNA from Patient 1 and her mother was isolated from blood using the TRIzol reagent, and reverse-transcribed (3μg) using MMLV reverse transcriptase (Invitrogen, La Jolla, CA). Complementary DNA (cDNA) was PCR-amplified with *STXBP1*-specific primers flanking intron-3 (contains the splicing mutation): STXBP1_ex2F: 5′AAGAAGAAGGGGGAATGGAA-3′ and STXBP1_ex4-5R: 5′-GAGAGTGGACGGACTTCTCG-3′, and with GAPDH-specific primers GAPDH_F: 5′-CCA CTCCTCCACCTTTGAC-3′ and GAPDH_R: 5′-ACCCTGTTGCTGTAGCCA-3′. The intensity of each PCR band was calculated using Quantity One software vs4.6.1 (BioRad, Mississauga, ON, Canada). Statistical analysis (n = 4) was conducted using a Student's *t* test. cDNA from Patient 1 and her mother were also amplified using forward primer STXBP1_ex3-mut-int3F:5′-AGGGCATAACGA**a**tgagca-3′, specific for the mutated allele (c.169+1G>A splicing mutation; underlined), and reverse primer STXBP1_ex4-5R. Resulting PCR products were subcloned into TOPO-TA PCR2.1 (Invitrogen) and sequenced.

Results

We identified three heterozygous mutations in *STXBP1* in three patients of our MR cohort. These mutations, including a missense (c.455C>G; p.S152C), a nonsense (c.1162C>T; p.R388X), and a mutation located in the consensus splice donor site of intron 3 (c.169+1G>A), were found in single patients. The missense c.455C>G is unlikely to be pathogenic because it is transmitted from a healthy father. In contrast, the splice junction c.169+1G>A (Patient 1) and the nonsense c.1162C>T (Patient 2) mutations were de novo (not present in blood DNA of the parents of the affected individuals) (Fig 1). Sequencing all *STXBP1* exons and intronic junctions in two control cohorts by our group (n = 190) and by the Sanger Institute's ExoSeq project (n = 48 Europeans; http://www.sanger.ac.uk/cgi-bin/humgen/exoseq/exoseqview?gene=STXBP1) did not show any splicing or amino acid–altering mutations. No amino acid–altering or splicing mutations were found in the other four sequenced genes *(STX1A, VAMP2, SNAP25, SYT1)* in the MR cohort.

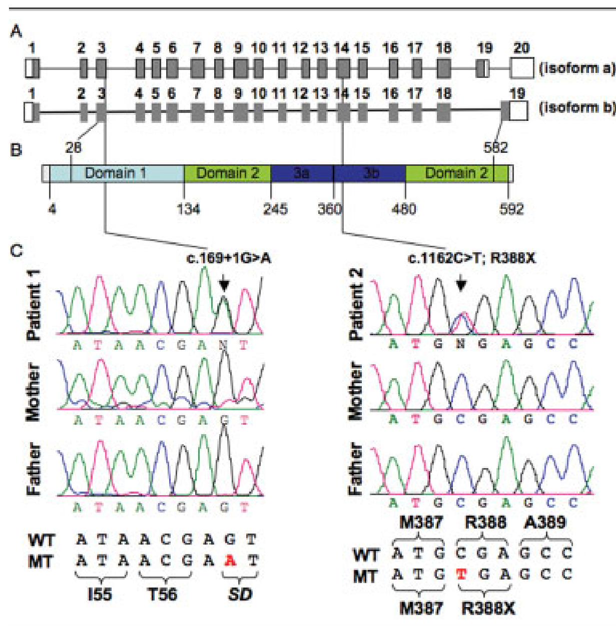

Fig 1. Location of the heterozygous de novo mutations of STXBP1. *(A) Schematic presentation of* STXBP1 *exon-intron regions (isoform a: NM_003165; isoform b, which lacks exon 19: NM_001032221);* filled boxes *represent coding exons, whereas* white boxes *indicate untranslated sequence. (B) Domain organization of STXBP1 based on the rat STXBP1 crystal structure.[9] Amino acids 28 to 582 represent SEC1 domain, an evolutionary conserved region domain present in Sec1/Munc18 family. (C) Families with de novo mutations in* STXBP1. *Chromatograms corresponding to the* STXBP1 *sequence for each patient and her parents are shown. Wild-type (WT) and mutant (MT)* STXBP1 *DNA sequences are shown together with the corresponding amino acids.* SD = *indicates consensus splice donor sequence. Coding nucleotide and amino acid positions are based on isoform-a of* STXBP1 *(Refseq NM_003165).*

To determine the impact of the de novo mutation c.169+1G>A on splicing, we performed reverse transcriptase PCR targeting exons flanking intron 3 of *STXBP1,* which contains this substitution, using total RNA isolated from the blood of Patient 1 and her mother (Fig 2A). Only one fragment (195bp), corresponding to the wild-type *STXBP1* allele, was amplified from the patient's and mother's cDNA. A longer fragment corresponding to the improperly spliced allele was not observed in the patient, possibly because its amplification may be difficult in the context of this PCR assay where both wild-type and mutant *STXBP1* cDNA compete for the same primers. Semiquantitative analysis indicated that the intensity of the wild-type band obtained from Patient 1 was approximately 50% of that from her mother (see Fig 2B). This observation is consistent with the possibility that this fragment was amplified from only one allele in the patient but from two alleles in her mother. We attempted to specifically amplify the mutant *STXBP1* transcript using a forward primer (ex3-mut-int3F) targeting the exon-3/intron-3 junction and containing the c.169+1G>A mutation at its 3′-end, as well as a reverse primer targeting the

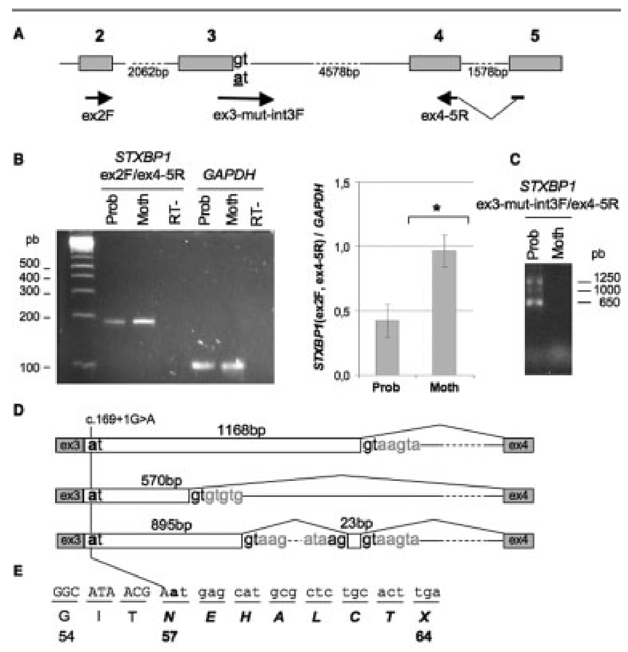

Fig 2. Characterization of the effect of the c.169+1G>A mutation on STXBP1 *splicing. (A) Schematic representation of the genomic region between exons 2 and 5 of* STXBP1. *The mutation at the beginning of intron three consensus donor site, c.169+1 G>A, is underlined, and the primers used for polymerase chain reaction (PCR) amplifications are represented by* arrows. *Primer ex4-5R spans two adjacent exons, ensuring that only complementary DNA (cDNA) would be amplified. (B) Agarose gel electrophoresis of reverse transcript polymerase chain reaction (RT-PCR) products from Patient 1 (Prob) and her mother (Moth) with* STXBP1 *primers ex2F/ex4-5R and* GAPDH*_F/R. To detect any possible contaminating genomic DNA, we also performed PCR on a negative control of reverse transcription (without reverse transcriptase [RT−]) with total RNA from the mother and Patient 1. The histogram shows the average ± standard error of the mean of band intensities obtained from PCR using ex2F and ex4-5R normalized by band intensities of the GAPDH amplification product (n = 4). *p < 0.001, Student's* t *test. (C) Agarose gel electrophoresis of products obtained by PCR on cDNA from Patient 1 and her mother using primers ex3-mut-int3F/ex4-5R, which amplify specifically the mutated allele. (D) Representation of the three main alternative splicing isoforms that occur in intron 3 when the normal splice donor is mutated (c.169+1G>A), as demonstrated by sequencing of the cloned PCR products shown in (C). Exons (ex) are represented by* gray boxes. *"at" represents the mutated splice donor site. Intronic sequences incorporated in the* STXBP1 *transcript because of the splicing mutation are depicted as* white boxes. *(E) Predicted translational consequence of c.169+1G>A mutation. Activation of various cryptic splice sites in intron 3, leading to transcripts retaining intronic sequences (shown in small letters) that are predicted to add, at position 57, an additional 7 amino acids followed by an inframe premature stop codon (shown in italics).*

Table. Clinical Features of Patients with De Novo Mutations in STXBP1

Characteristics	Patient 1	Patient 2
De novo mutation	c.169=1G>A	c.1162C>T/ p.R388X
Age, yr	27	15
Sex	F	F
Ethnic origin	French Canadian	French Canadian
Severe mental retardation	+	+
Epilepsy		
Age at first seizures	6 weeks	2 years 9 months
Initial type of seizures	Partial complex	Partial complex
Initial electroencephalogram	Focalized	Focalized
Neurological examination		
Head circumference (centile)	56cm (75th)	55.7cm (50-75th)
Hypotonia	+	+
Tremor	+	+
Hyperventilation	+	–
Brain imaging		
Magnetic resonance imaging	Not done	Normal
Computed tomographic scan	Normal	Normal

junction of exons 4 and 5 (ex4-5R) (see Fig 2A). Different PCR products of approximately 650, 1,000, and 1,250bp were amplified from the cDNA of Patient 1, but no product was amplified from her mother's (see Fig 2C). These products were cloned and sequenced, demonstrating the incorporation of intronic sequences into the *STXBP1* transcript presumably because of the activation of different cryptic splice sites in intron 3 (see Fig 2D). All the corresponding transcripts result in the insertion of seven new amino acids at position 57 followed by a premature stop codon (see Fig 2E).

The splicing mutation in Patient 1 is predicted to truncate STXBP1 in the domain-1 region (see Fig 1), which is implicated in binding to syntaxin-1.[5,9] Binding of STXBP1 to syntaxin-1 and the SNARE complex is essential for SNARE complex assembly and subsequent neurotransmitter release.[5,10–12] Similarly, the p.R388X mutation in Patient 2 is also predicted to truncate STXBP1. This stop codon lies in domain 3 of STXBP1 (see Fig 1), which together with domain 1 form the central cavity providing binding surfaces for syntaxin-1.[9]

The two patients with de novo mutations showed severe MR with hypotonia, a particular gait, tremor, and epilepsy (Table; see supplementary file). Patient 1 had her first seizures, described as loss of visual contact, upward gaze deviation followed by clonic movements of the face, when she was 6 weeks old. Her initial electroencephalogram (EEG) showed epilepsy in the right frontotemporal region. Phenobarbital administration immediately controlled the seizures and normalized the EEG. Seizures did not recur over the next 2 years and the medication was stopped. She was seizure free until 8 years of age when she had two generalized seizures. Over the years, she continued to suffer from recurrent seizures despite the administration of several antiepileptic drugs. She currently has three seizures per day, including generalized tonic seizures beginning by a shout, drop attacks, and complex partial seizures with staring. Patient 2 first showed left versive seizures at 2 years and 9 months of age, with an epileptic focus in the right temporal region. Seizures were well controlled for the next 2 years with carbamazepine. She then had right versive seizures and clobazam was added. Since then, she had one or two seizures every 6 months. These seizures are now motor seizures on the right side and occur exclusively at night.

Because MR and ASD tend to coexist in the same patients, we also sequenced *STXBP1* in a cohort of 142 cases of ASD. No de novo, splicing, or truncating mutations were found in the ASD cohort. The number of *STXBP1* de novo mutations in our MR cohort was significantly greater ($p < 0.05$, Fisher's exact test) than in the rest of the tested cohorts (our control subjects, ExoSeq control subjects, and ASD cases). The only amino acid–altering mutation found was the heterozygous missense variant c.1302G>A (p.R536H), which lies in a domain that does not interact with syntaxin-1.[9] This mutation is unlikely to be pathogenic because it is transmitted from an unaffected parent.

Discussion

We identified de novo mutations in the autosomal gene *STXBP1* in two patients with similar clinical pictures including severe MR, hypotonia, and epilepsy. These mutations are likely to be pathogenic for several reasons. First, both the c169+1G>A and p.R388X mutations are predicted to truncate STXBP1 toward the beginning and middle (see Fig 1), respectively, resulting in a protein lacking a substantial portion of the domains of interaction with syntaxin-1 (domains 1/3)[9] (see Fig 1). Second, although *Stxbp1* homozygous deletion results in complete loss of neurotransmission and postnatal lethality, heterozygous mice survive but display impaired neurotransmission,[13,14] suggesting that disruption of a single allele in humans is also likely to alter synaptic transmission. Similarly, studies done on *STXBP1* orthologues in *Drosophila (rop)* and *C. elegans (unc-18)* showed that truncating point mutations, situated in similar or even downstream positions to the de novo mutations reported in this study, cause defects in synaptic vesicle docking and neurotransmission.[15–17] Third, screening of 380 individuals belonging to control and ASD cohorts failed to identify any splicing, truncating, or de novo amino acid–altering mutations. Finally, Saitsu and colleagues[18] recently described four heterozygous missenses (three de novo) in *STXBP1,* including one mutation that reduced binding to syntaxin-1A, in patients with early infantile epileptic encephalopathy (EIEE), also called *Ohtahara syndrome.* These authors also described a de novo deletion encompassing *STXBP1* and 38 other genes in a patient with EIEE. Collectively, these results suggest that loss of *STXBP1* function is associated with epilepsy and MR.

The patients that Saitsu and colleagues[18] described showed the typical features of EIEE, including onset of seizure during the neonatal period, tonic spasms, initial presence of suppression bursts on the EEG, transition toward West syndrome and hypsarrhythmia, as well as a poor outcome with intractable seizures and severe MR.[19] Although the patients described in our study also suffer from severe MR and epilepsy, they were not suspected to have EIEE. They never had the typical tonic seizures or infantile spasms associated with EIEE and West syndromes, respectively. Seizures started after 2 years of age in one patient and in both cases were initially easy to control. Abnormal EEGs suggesting burst suppression or hypsarrhythmia were never observed in these patients. EEGs were initially focalized and rapidly became normal. With time they changed to show a pattern that could evoke Lennox–Gastaut syndrome, which can be later observed in patients with EIEE, but none of the patients showed the atypical absence or tonic nocturnal seizures associated with this syndrome. Indeed, our patients did not show any consistent pattern of epileptic features. Our observation thus indicates that *STXBP1* disruption has the potential of causing a wide spectrum of epileptic disorders in association with severe MR.

Interestingly, disruption of another synaptic vesicle protein that also modulates neurotransmission, synapsin-1/*SYN1,* has been previously described to cause epilepsy and learning disability.[20] This observation and the documentation of mutations in *STXBP1* raise the possibility that mutations in other genes implicated in synaptic vesicle exocytosis and neurotransmission may be similarly involved in forms of MR and epilepsy. Although no deleterious mutations were identified in the other studied genes implicated in such pathways (*STX1A, VAMP2, SNAP25,* and *SYT1*), screening of larger cohorts would be needed before ruling out their involvement in MR and epilepsy. In addition, our work provides some validation for the use of a candidate gene approach to explore the involvement of such synaptic pathways.

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References

1. Chelly J, Khelfaoui M, Francis F, et al. Genetics and pathophysiology of mental retardation. Eur J Hum Genet 2006;14:701–713.
2. Zoghbi HY. Postnatal neurodevelopmental disorders: meeting at the synapse? Science 2003;302:826–830.
3. Rizo J, Rosenmund C. Synaptic vesicle fusion. Nat Struct Mol Biol 2008;15:665–674.
4. Wojcik SM, Brose N. Regulation of membrane fusion in synaptic excitation-secretion coupling: speed and accuracy matter. Neuron 2007;55:11–24.
5. Burkhardt P, Hattendorf DA, Weis WI, Fasshauer D. Munc18a controls SNARE assembly through its interaction with the syntaxin N-peptide. Embo J 2008;27:923–933.
6. Chicka MC, Hui E, Liu H, Chapman ER. Synaptotagmin arrests the SNARE complex before triggering fast, efficient membrane fusion in response to Ca2+. Nat Struct Mol Biol 2008;15:827–835.

7. Fisher RJ, Pevsner J, Burgoyne RD. Control of fusion pore dynamics during exocytosis by Munc18. Science 2001;291: 875–878.
8. Gerber SH, Rah JC, Min SW, et al. Conformational switch of syntaxin-1 controls synaptic vesicle fusion. Science 2008;321: 1507–1510.
9. Misura KM, Scheller RH, Weis WI. Three-dimensional structure of the neuronal-Sec1-syntaxin 1a complex. Nature 2000; 404:355–362.
10. Dulubova I, Khvotchev M, Liu S, et al. Munc18–1 binds directly to the neuronal SNARE complex. Proc Natl Acad Sci U S A 2007;104:2697–2702.
11. Gulyas-Kovacs A, de Wit H, Milosevic I, et al. Munc18–1: sequential interactions with the fusion machinery stimulate vesicle docking and priming. J Neurosci 2007;27:8676–8686.
12. Khvotchev M, Dulubova I, Sun J, et al. Dual modes of Munc18–1/SNARE interactions are coupled by functionally critical binding to syntaxin-1 N terminus. J Neurosci 2007;27: 12147–12155.
13. Toonen RF, Wierda K, Sons MS, et al. Munc18–1 expression levels control synapse recovery by regulating readily releasable pool size. Proc Natl Acad Sci U S A 2006;103:18332–18337.
14. Verhage M, Maia AS, Plomp JJ, et al. Synaptic assembly of the brain in the absence of neurotransmitter secretion. Science 2000;287:864–869.
15. Sassa T, Harada S, Ogawa H, et al. Regulation of the UNC-18-Caenorhabditis elegans syntaxin complex by UNC-13. J Neurosci 1999;19:4772–4777.
16. Weimer RM, Richmond JE, Davis WS, et al. Defects in synaptic vesicle docking in unc-18 mutants. Nat Neurosci 2003;6: 1023–1030.
17. Wu MN, Littleton JT, Bhat MA, et al. ROP, the Drosophila Sec1 homolog, interacts with syntaxin and regulates neurotransmitter release in a dosage-dependent manner. Embo J 1998;17: 127–139.
18. Saitsu H, Kato M, Mizuguchi T, et al. De novo mutations in the gene encoding STXBP1 (MUNC18–1) cause early infantile epileptic encephalopathy. Nat Genet 2008;40:782–788.
19. Ohtahara S, Yamatogi Y. Ohtahara syndrome: with special reference to its developmental aspects for differentiating from early myoclonic encephalopathy. Epilepsy Res 2006;70(suppl 1): S58–S67.
20. Garcia CC, Blair HJ, Seager M, et al. Identification of a mutation in synapsin I, a synaptic vesicle protein, in a family with epilepsy. J Med Genet 2004;41:183–186.

Free Sialic Acid Storage Disease without Sialuria

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We performed high-resolution in vitro proton nuclear magnetic resonance spectroscopy on cerebrospinal fluid and urine samples of 44 patients with leukodystrophies of unknown cause. Free sialic acid concentration was increased in cerebrospinal fluid 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.

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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

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