Objective: To characterize a novel SCN1A mutation in a proband with malignant migrating partial seizures of infancy.

Design: Genomic DNA was isolated from blood and submitted for commercial testing. The identified missense mutation was confirmed in brain DNA obtained at autopsy. Genomic DNA from the brain of the proband was analyzed by comparative genome hybridization, and the coding exons of SCN9A were amplified. Quantitation studies of the mutant transcript were performed.

Setting: Children’s National Medical Center and Yale University School of Medicine.

Proband: A full-term female infant who experienced seizure onset at age 10 weeks, with progression of hemi-clonic, apneic, and multifocal migrating partial seizures leading to recurrent status epilepticus and death at age 9 months.

Main Outcome Measures: Electroencephalographic and magnetic resonance imaging results, quantitative RNA expression, and secondary mutation test results.

Results: The heterozygous missense mutation c.C5006C>A was identified by sequencing genomic DNA from blood and was confirmed in brain DNA. The resulting amino acid substitution p.A1669E alters an evolutionarily conserved residue in an intracellular linker of domain 4 of the SCN1A sodium channel protein Na1.1. The mutant transcript is found to be expressed at levels comparable to the wild-type allele in brain RNA. No variation in copy number was detected in the chromosome region 2q24 containing SCN1A or elsewhere in the genome. No mutations were detected in the linked sodium channel gene SCN9A, which has been reported to act as a modifier of SCN1A mutations.

Conclusion: This report expands the spectrum of SCN1A epileptic channelopathies to include malignant migrating partial seizures of infancy.

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INCE ITS INITIAL DESCRIPTION in 1995, the syndrome of malignant migrating partial seizures of infancy has become increasingly well recognized, with approximately 50 cases reported worldwide. The main features are normal development before seizure onset, seizure onset before 6 months of age, migrating focal motor seizures at onset with progression to multifocal seizures that are intractable to conventional antiepileptic drugs, and generally unfavorable outcome, with death usually occurring in the first 1 to 2 years of life. The cause remains elusive despite extensive neurometabolic, biochemical, and neuroradiologic investigations. Certain features of the syndrome suggest a genetic basis, including early age at onset, progressive psychomotor retardation, acquired microcephaly, and multifocality of seizures. Prior studies3,4 of candidate genes associated with other infantile epilepsy syndromes failed to detect mutations. We now describe a novel mutation in the SCN1A (OMIM *182389) sodium channel in an infant who met clinical and electroencephalographic criteria for the syndrome of malignant migrating partial seizures of infancy.

METHODS

ELECTROENCEPHALOGRAPHY

Continuous digital video electroencephalographic recordings were acquired using the internationally standard 10-20 Electrode Placement System on several occasions, the lengths of which ranged from 30 minutes to several days. The recordings were then analyzed at 24-hour increments and were interpreted by board-certified staff clinical neurophysiologists (W.D.G., J.A.C., T.N.T., and P.L.P.).

MAGNETIC RESONANCE IMAGING

Magnetic resonance imaging (MRI) results were obtained using a 1.5-T GE scanner (GE Healthcare, Buckinghamshire, England) with a standard infant epilepsy protocol, including sagittal and axial T1 images, axial...
diffusion-weighted images, axial spin-echo proton density and T2-weighted images, coronal fast spin-echo T2 images with fat saturation, coronal high-resolution T2 images, and sagittal fast spin-echo T2 images at 3 separate time points (2 months, 5 months, and 7 months of age). Single-voxel short echo time and long echo time spectroscopic imaging was performed in the left basal ganglia of the proband at age 7 months.

**HIGH-RESOLUTION KARYOTYPE AND COPY NUMBER VARIATION**

Peripheral blood from the proband was analyzed at a banding level of 650 G-bands or more. Genomic DNA from proband brain cells and a DNA strand from a female control individual were analyzed by comparative genome hybridization with the 244 K Agilent array in the Molecular Medical Genetics Core at the University of Michigan (Ramaswamy Iyer, PhD, oral communication, January 8, 2010), as described herein.

**SCN1A AND SCN9A SEQUENCES**

Genomic DNA was isolated from blood and submitted for commercial testing by sequence of exons and splice sites (Athena Diagnostics Inc, Worcester, Massachusetts). The missense mutation in exon 26 was confirmed by polymerase chain reaction (PCR) amplification from phenol-chloroform–extracted DNA obtained from the brain at autopsy, followed by gel purification and sequencing, as previously described. Total RNA was prepared from brain tissue obtained at autopsy (22 hours after death) using Trizol Reagent (Invitrogen Corp, Carlsbad, California). Reverse transcription (RT)–PCR products were sequenced in the University of Michigan Sequencing Core. The coding exons of SCN9A were amplified from brain genomic DNA. Sequencing was then performed, as previously described.

QUANTITATION OF THE MUTANT TRANSCRIPT

A 571-base pair (bp) fragment of the SCN1A complementary DNA containing the proband’s mutation was amplified by RT-PCR from brain RNA using a 23-mer forward probe end-labeled with the fluorescent dye 6-FAM (Eurofins MWG Operon, Huntsville, Alabama). The product was digested with the endonuclease Ddel (New England Biolabs Inc, Ipswich, Massachusetts), and the resulting fragments were separated on an automated sequencer (3730 DNA Analyzer; Applied Biosystems Inc, Foster City, California). The fluorescent-labeled 5’ end fragments were quantitated using GeneMapper, version 4.0 (Applied Biosystems Inc) and Genemarker (Softgenetics LLC, State College, Pennsylvania) software in the University of Michigan Sequencing Core.

RESULTS

CLINICAL FEATURES

The proband was a female infant (the firstborn of twins) born at 37.5 weeks, whose conception had resulted from in vitro fertilization with a donor ovum. No history of seizures was documented in the family of the father or that of the ovum donor. A paternal half-brother had had normal development but had died at age 4 years; his diagnosis was sudden unexplained death in childhood.

The proband had mild hypotonia within the first month of life with otherwise normal early development. Seizure onset at 10 weeks of age was manifested by left arm clonic activity of 3 hours’ duration despite multiple doses of lorazepam. Five days later, the proband had a clonic seizure of her right arm, which responded to a loading dose of phenobarbital. Results of routine analysis of electrolytes, calcium, and glucose levels were normal. The initial electroencephalographic recording showed prolonged sleep spindles on an otherwise normal background, and brain MRI results were normal. The proband remained clinically seizure free for 21/2 months while taking phenobarbital.

At 5 months of age, the proband developed recurrent episodic status epilepticus that was refractory to conventional therapy. Seizure characteristics were hemi-clonic, multifocal clonic, and apneic. Status epilepticus recurred on average every 5 to 8 days. A 5-week seizure-free interval occurred while the proband was being treated with supratherapeutic levels of phenobarbital (levels of 60-90 µg/mL), valproic acid, fosphenytoin sodium, topiramate, levetiracetam, and lorazepam. Her seizures returned when she was aged 7 months and they became increasingly refractory to intervention. She developed profound encephalopathy and became progressively unresponsive. Her head circumference decelerated from the mean at age 2 months to the second percentile by age 9 months. Despite therapy with several pentobarbital infusions and trials of clobazam, rufinamide, stiripentol, vigabatrin, felbamate, high-dose corticosteroids, and intravenous immunoglobulin, the proband deteriorated clinically and died at age 9 months. Microcephaly was confirmed on autopsy, with a brain weight of 613 g, compared with an expected 750 g.

ELECTROPHYSIOLOGY AND IMAGING

The electroencephalographic recordings obtained during episodes of status epilepticus initially suggested seizure onset from the right posterior hemisphere. Within days, a transition originated in the left posterior hemisphere and subsequently broadened to multifocal onset (Figure 1). Over time, the ictal and interictal recordings became indistinguishable, ultimately showing virtually continuous migratory ictal foci (Figure 2).

When the proband was aged 5 months, MRI scans revealed restricted diffusion of the right hippocampus, which did not persist on follow-up and was attributed to peri-ictal activity. A subsequent MRI scan at 7 months demonstrated absence of callosal growth and myelination, as well as cerebral atrophy. Magnetic resonance spectroscopy using a single voxel over the basal ganglia suggested decreased N-acetylaspartate levels (Figure 3).

BIOCHEMICAL ANALYSIS

No abnormalities were detected by metabolite analysis that included plasma amino acids, urine organic acids, piperolic acid, plasmalogens, fatty acids, acylcarnitine profile, isoelectric focusing of transferrin, and cerebrospinal fluid analysis of neurotransmitters, lactate, and amino acids. Results of DNA methylation analysis for Angelman syndrome did not detect abnormalities.

GENOMEWIDE MUTATION ANALYSIS

The high-resolution karyotype was normal. Copy number analysis by comparative genome hybridization using the Affymetric 244K chip (Affymetric, Santa Clara, California) did not detect any novel deletions or insertions.

MUTATION OF SODIUM CHANNEL SCN1A

SCN1A gene sequencing from blood DNA was obtained from the proband at age 5 months because of her initial presentation with frequent hemiclonic status epilepticus. A heterozygous cytosine to adenine transversion, c.C5006C>A, was detected. The mutation was confirmed in brain DNA obtained post mortem (Figure 4A). Paternal SCN1A test results were negative; maternal DNA was unavailable.

The mutation is predicted to change the hydrophobic amino acid alanine at residue 1669 to the acidic residue glutamic acid (p.A1669E). Alanine 1669 is located within a short cytoplasmic linker between transmembrane segments 4 and 5 of domain 4 of the sodium channel (Figure 4B). Alanine 1669 is evolutionarily conserved in the human paralogs of SCN1A and in vertebrate and invertebrate sodium channel genes (Figure 4C). The evolutionary conservation, together with the alteration in chemical properties, strongly predicts that A1669E is a pathogenic variant. This theory is supported by analysis with the protein prediction programs PolyPhen (http://genetics.bwh.harvard.edu/pph/) and SIFT (http://sift.jcvi.org/), which predict that A1669E is highly likely to be a deleterious mutation. Three other pathologic mutations located in the same linker region have previously been reported.

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EXPRESSION OF MUTANT AND WILD-TYPE SCN1A TRANSCRIPTS IN THE HETEROZYGOUS PROBAND

Brain RNA was amplified by RT-PCR. The overall abundance of the SCN1A transcript in proband and control brain matter was comparable (Figure 5A). Sequencing of the RT-PCR product of the proband demonstrated that the mutant transcript is expressed (not shown). To quantify the relative expression of mutant and wild-type (WT) transcripts, we developed an assay based on the introduction of a novel Ddel restriction site (CTNAG) by the c.C5006C>A mutation, which changes the WT sequence CTGCG to the Ddel consensus CTGAG (Figure 4A). A 571-bp RT-PCR product containing the mutation was 5’ end-labeled with the fluorescent tag 6-FAM. Digestion of the labeled PCR product with Ddel generated 5’ fragments of 528 bp from the WT transcript and 280 bp from the mutant transcript (Figure 5A and B). These fragments were size-separated on an automated DNA sequencer and detected by fluorescence (Figure 5C). Quantitation of the areas corresponding to
the 2 fragments demonstrated a ratio of approximately 60:40 of WT to mutant transcript (Figure 5D). This result demonstrates that the wild-type allele is expressed in the proband, eliminating the possibility of an unrecognized regulatory mutation that could reduce overall channel expression. In addition, the stability of the mutant transcript is demonstrated.

SEQUENCE OF SCN9A

Amplification and sequencing of the exons and adjacent splice sites of SCN9A from brain DNA detected no novel variants of SCN9A affecting amino acid sequence or splice sites. The proband is heterozygous for the polymorphic variant R1150W, which has an allele frequency of approximately 0.15 and may be associated with hypersensitivity to pain.17,18

The clinical course of our proband is more severe than the typical course of epilepsy caused by mutations of SCN1A and fulfills the original proposed criteria for malignant migrating partial seizures in infancy: normal development before seizure onset, seizure onset before age 6 months, migrating focal motor seizures, multifocal seizures that became intractable and refractory to therapy, and profound psychomotor delay.1-8 Other typical features include prominent apneic seizures, acquired microcephaly, and decreased N-acetylaspartate as quantified by spectroscopy.1,9 The electroencephalographic recording evolved to exhibit multiple foci, characteris-

tic overlap of ictal and interictal recordings, and migratory ictal foci. The migratory pattern is described as rhythmic theta activity beginning in 1 region and progressively involving adjacent areas, with independent discharges appearing in other regions although the original patterns persist or fade, producing complex electrophyslogic multifocal status epilepticus.2 Our proband meets the clinical and electroencephalographic diagnostic criteria for this malignant syndrome, for which no cause has been elucidated. Mutational analysis of SCN1A had been performed in 3 prior patients with malignant migrating partial seizures in infancy, and no mutations were detected.9

We identified a novel mutation affecting an evolutionarily conserved amino acid residue of the sodium channel protein Na,1.1 encoded by the gene SCN1A. More than 600 mutations of SCN1A have been reported, most of them in patients with the sporadic syndrome severe myoclonic epilepsy of infancy, also known as Dravet syndrome.15,16,19,20 Mutations also have been reported in patients with the related syndrome severe myoclonic epilepsy borderland, a variant that lacks the generalized spike-wave and myoclonus of Dravet syndrome, and in the typically milder inherited epilepsy syndrome known as generalized epilepsy with febrile seizures plus other seizure types (GEFS+).14,15,20 Most variants associated with the more severe phenotypes occur de novo, in contrast

Figure 3. Brain magnetic resonance imaging (MRI) scans and magnetic resonance spectroscopy (MRS) results from 2 to 7 months of age demonstrating lack of appropriate growth. A, MRI at 2 months of age. B, MRI at 5 months of age. C, MRI at 7 months of age with progressive atrophy, lack of myelination, and ventriculomegaly. D, MRS at 7 months demonstrating equal levels of choline and N-acetylaspartate, suggestive of low N-acetylaspartate levels.

Figure 4. Proband mutation A1669E in SCN1A. A, DNA sequence from brain. B, Position of mutation in the sodium channel protein. C, Evolutionary conservation of the mutated residue.
to the autosomal dominant inheritance pattern with variable penetrance seen in GEFS+.

The spectrum of SCN1A-associated infantile epileptic encephalopathies also has been reported to include a syndrome described as severe infantile multifocal epilepsy.20 SCN1A mutations were identified in 3 of 5 patients with this disorder, although the phenotype appears to be milder than malignant migratory partial seizures of early infancy because developmental slowing occurred between the ages of 16 months and 6 years, and the patients were studied between the ages of 5 and 20 years. Difficulty often occurs with respect to classifying patients with cryptogenic epilepsies that begin in the first year of life.21,22 Patients with severe myoclonic epilepsy in infancy may initially be classified as having cryptogenic localization-related epilepsy, which may lead to misguided surgical procedures.

The phenotype of the proband described herein was more severe than that typically seen in individuals with severe myoclonic epilepsy of infancy, many of whom are heterozygous for mutations that cause complete loss of function of SCN1A.24 The novel amino acid substitution A1669E in our proband changes the charge of a short cytoplasmic linker joining the S4 and S5 transmembrane segments in domain 4 of the channel (Figure 4). This 12-residue linker is the site of 3 previously described mutations in patients with severe myoclonic epilepsy borderline or severe myoclonic epilepsy of infancy: T1658R, F1661S, and P1668A.14-16 The functional effects of these substitutions on Na,1.1 channel function have not been determined. However, the pathogenic mutation A1632E introduces the identical amino acid substitution into the corresponding residue of SCN9A.23 This SCN9A mutation was identified in a patient with a complex pain disorder. Functional characterization of this mutation in SCN9A revealed multiple biophysical abnormalities, including a 7-mV hyperpolarizing shift in the voltage dependence of channel activation.24 In addition, the pathogenic mutation A1071T substitutes a polar residue into the S4-S5 linker of domain 3 in mouse SCN8A and results in a 14-mV shift in voltage dependence of activation.24 The biophysical alterations resulting from these mutations in closely related sodium channels support the pathogenicity of the SCN1A mutation in our proband.

We performed several experiments to attempt to explain the severe phenotype associated with the A1669E mutation. Using comparative genome hybridization, we found no unusual copy number variant in our proband. The severity of SCN1A mutations has been reported to be exacerbated by deleterious variants in the closely linked sodium channel gene SCN9A.25 Because of the clinical severity of the proband’s disease, we tested the possibility of an additional mutation in SCN9A, and no such mutation was identified. Quantitative analysis of brain RNA demonstrated that wild-type and mutant alleles are expressed at comparable levels, indicating that no deficiency of wild-type protein exists. The mutant protein could have a dominant negative effect on the wild-type channel, but such effects of mutant sodium channels only rarely have been observed.26 Other potential explanations for the severe phenotype in this proband include unidentified factors in her genetic background, such as variation in other ion channels27 or stochastic processes that can exacerbate the effects of any mutation during development.

This case extends the spectrum of SCN1A-associated epileptic encephalopathies to a more severe phenotype and suggests a cause for the likely heterogeneous yet elusive syndrome of malignant migrating partial seizures of infancy. Therefore, SCN1A sequencing is recommended in individuals with the phenotype of malignant migrating partial seizures of infancy.

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**Figure 5.** Quantitation of mutant and wild-type (WT) SCN1A transcripts in proband brain RNA. A, Two novel Ddel products of 280 and 247 base pairs (bp) in the heterozygous proband. The total abundance of the polymerase chain reaction product is similar in the proband and control (−Ddel) lanes. B, Strategy for quantitation of mutant and WT transcripts in heterozygous proband RNA based on the new Ddel site generated by the C→A mutation. C, Capillary electrophoretic separation of the 5′ end-labeled Ddel fragments of 528 bp (WT allele) and 280 bp (mutant allele) in the reverse transcription-polymerase chain reaction product from the proband. D, Quantitation of the 280-bp and 528-bp products by calculation of peak area (2326 and 3370 arbitrary units, respectively).


