

# Recurrent De Novo Mutations of *SCN1A* in Severe Myoclonic Epilepsy of Infancy

Jennifer A. Kearney, PhD\*, Anna K. Wiste, BS<sup>†</sup>, Ulrich Stephani, MD<sup>‡</sup>,  
Michelle M. Trudeau, MS\*, Anne Siegel, BS<sup>†</sup>, Rajesh RamachandranNair, MD<sup>§</sup>,  
Roy D. Elterman, MD<sup>||</sup>, Hiltrud Muhle, MD<sup>‡</sup>, Juliane Reinsdorf, MD<sup>‡</sup>,  
W. Donald Shields, MD<sup>||</sup>, Miriam H. Meisler, PhD\*, and Andrew Escayg, PhD<sup>†</sup>

Mutations in the voltage-gated sodium channel gene *SCN1A* are a major cause of severe myoclonic epilepsy of infancy (Dravet syndrome) and generalized epilepsy with febrile seizures plus. This study reports the identification of six *de novo* *SCN1A* mutations in patients with severe myoclonic epilepsy of infancy, including a tetranucleotide deletion in exon 26. The same deletion was previously observed in two unrelated patients and appears to result from slipped-strand mispairing of a direct repeat during deoxyribonucleic acid replication. Review of the literature indicates that recurrent mutations account for 25% of *SCN1A* mutations in severe myoclonic epilepsy of infancy, including six sites of deamination at CpG dinucleotides. © 2006 by Elsevier Inc. All rights reserved.

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## Introduction

Mutations in the voltage-gated sodium channel *SCN1A* are responsible for inherited epilepsy with a broad spectrum of clinical severity. Our initial report in 2000 described *SCN1A* mutations in two families with generalized epilepsy with febrile seizures plus (GEFS+) (OMIM 604233), a usually mild seizure disorder [1]. At the present time, more than 150 *SCN1A* mutations have been identified, most of them in

patients with severe myoclonic epilepsy of infancy (SMEI) or Dravet syndrome (OMIM 607208) [2]. Ninety percent of severe myoclonic epilepsy of infancy mutations arise *de novo* in affected individuals [3-5]. Approximately 50% of these mutations result in truncated proteins, demonstrating haploinsufficiency for *SCN1A*, and several of the missense mutations in patients with severe myoclonic epilepsy of infancy also appear to cause loss of function [6]. Several distinct biophysical mechanisms have been associated with mutations in generalized epilepsy with febrile seizures plus, including impaired channel inactivation and reduced time spent in the inactivated state, both of which lead to increased persistent sodium current at the cellular level. A unique mechanism was observed for the missense mutation D1866Y in the C-terminal domain, which impairs interaction with the sodium channel  $\beta$  subunit [7]. The current paper describes the identification of five new *SCN1A* mutations and one recurrent mutation in patients with severe myoclonic epilepsy of infancy. The deoxyribonucleic acid (DNA) sequence context at the site of each recurrent mutation is also examined.

## Methods

### Patients

Six patients were selected for analysis on the basis of their clinical presentation. Five patients were consistent with the diagnosis of severe myoclonic epilepsy of infancy according to the International League Against Epilepsy guidelines [8]. Patient 4 was consistent with borderline severe myoclonic epilepsy of infancy (SMEB) [9]. The patients were identified at clinics in three different countries, Germany (n = 3), Canada (n = 1), and the United States (n = 2).

The first two authors contributed equally to this work.

From the \*Department of Human Genetics, University of Michigan, Ann Arbor, Michigan; <sup>†</sup>Department of Human Genetics, Emory University, Atlanta, Georgia; <sup>‡</sup>Neuropediatric Department, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany; <sup>§</sup>Department of Pediatrics, The Hospital for Sick Children, Toronto, Canada; <sup>||</sup>Department of Pediatrics, University of Texas Southwestern Medical School at Dallas, Dallas, Texas; and <sup>¶</sup>Department of Pediatrics, University of California at Los Angeles.

Communications should be addressed to:  
Dr. Escayg; Department of Human Genetics; Emory University; 615 Michael Street; Whitehead Building, Suite 301; Atlanta, GA 30322.  
E-mail: aescayg@genetics.emory.edu  
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**Table 1. Clinical features and *SCN1A* mutations in six patients**

	Patient					
	1	2	3*	4	5	6
Sex	F	M	M	M	F	M
Age (yr)	5	2	15	20	18	2
First seizure						
Age (months)	5	4	13	10	16	7
Seizure type	PFS	GTCS	PFS	GTCS	FS	FS
Other seizures						
a) Myoclonic	+	+	+	–	–	+
b) GTCS	+	+	+	+	+	+
c) Other types	AS	AS, hemiclonic	Atypical AS, tonic	–	Atypical AS	CPS
EEG	3–4 Hz SWD	?	General slowing, irregular SWD	3–4 Hz SWD, irregular spikes	Generalized theta waves	Bifrontal spikes
MRI						
Age (years)	1	2	3	4	2 and 12	1
Result	Normal	Increased T <sub>2</sub> -weighted signal intensity in left medial temporal lobe	Normal	Slightly dilated lateral ventricles	Normal	Normal
Mental retardation	+	+	+	+	+	+
Ataxia	+	?	+	+	+	–
Mutation	P707fsX715	K1846fsX1856	S1231T	S914fsX934	R1525X	R613X
Exon	12	26	18	15	24	11
Nucleotide	2118insAA	5536-5539 delAAAC	G3692C	2740delA	C4573T	C1837T
Location in Nav1.1 protein	D1–D2 loop	C-terminus	D3S1	D2S5–D2S6	D3–D4 loop	D1–D2 loop
De novo	+	+	+	+	+	+

–, absent; +, present; ?, unknown.

\* Patient 3 also has cystic fibrosis (homozygous for the mutation  $\Delta F508$  in *CFTR*).

#### Abbreviations:

AS = Absence seizures

CPS = Complex partial seizures

FS = Febrile seizures

GTCS = Generalized tonic clonic seizures

PFS = Prolonged febrile seizures

SWD = Spike-wave discharges.

## DNA Isolation and Analysis

DNA was extracted from blood samples, and the 26 coding exons of *SCN1A* were amplified by polymerase chain reaction as previously described [1]. Gel purified fragments were sequenced using an ABI Model 3730 automated sequencer. When the sequencing results were suggestive of an insertion or deletion, heterozygosity was confirmed by cloning polymerase chain reaction products into pGEM-T Easy vector (Promega) and sequencing 10 clones using T7 and SP6 primers. Nucleotide and amino acid residue numbers correspond to the full-length *SCN1A* complementary DNA isoform containing the long form of exon 10 (Genbank Accession AB093548). Parental identity was confirmed by genotyping microsatellite markers from multiple chromosomes.

## Results

### Clinical Data

The clinical features of the six patients are summarized in Table 1. A family history of epilepsy was only observed for Patient 2. The father and mother of Patient 2 had febrile seizures and absence epilepsy, respectively. Consanguinity was not present in any of the families.

Because severe myoclonic epilepsy of infancy is often refractory to pharmacologic intervention, we reviewed the

medical records of each patient to determine which anti-epileptic medications were most beneficial in reducing seizure frequency. Treatment regimens varied between the different centers from which the patients were recruited, and little consistency was observed in response to anti-epileptic medications. Based on empirical impressions of seizure reduction, Patients 1 and 2 were refractory to all medications including valproate, topiramate, and phenobarbital. Strictly drug-monitored bromide therapy resulted in the disappearance of the generalized tonic clonic seizures in Patients 3 and 4. Lamotrigine was associated with a significant reduction in seizure frequency in Patient 5. Some reduction in seizure frequency was achieved with topiramate and phenobarbital in Patient 6.

### Genetic Analysis

Frameshift mutations of *SCN1A* were identified in three patients. In Patient 1 a dinucleotide insertion, 2118insAA, in exon 12 results in the frameshift mutation, 707fsX715, truncating the channel protein in the cytoplasmic loop between domains 1 and 2 (Fig 1A). In patient 4 a single

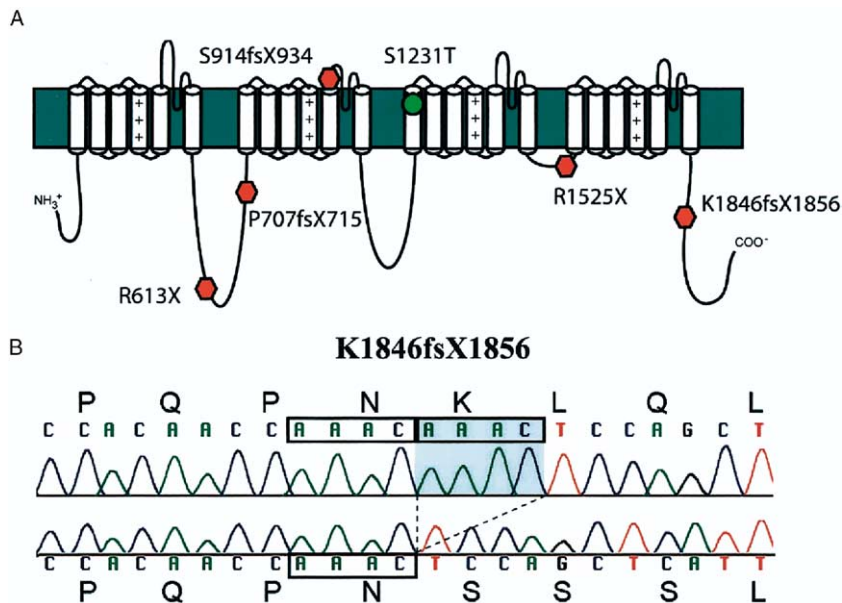


Figure 1. Identification of *SCN1A* mutations in patients with severe myoclonic epilepsy of infancy. (A) Positions of six identified mutations in the Nav1.1 channel protein. (B) Deletion of one copy of the directly repeated tetranucleotide AAAC in exon 26 of the mutated allele in Patient 2. Sequences of the two cloned alleles are depicted.

base deletion in exon 15 results in truncation of the channel protein in the pore region between D2S5 and D2S6 (Fig 1A). Analysis of Patient 2 identified a 4 bp deletion 5536-5539 $\Delta$ AAAC in exon 26 (Fig 1B). The resulting frameshift mutation, K1846fsX1856, truncates the final 153 residues of the cytoplasmic C-terminal domain of the channel (Fig 1A). Nonsense mutations in Patients 5 and 6 will result in channel proteins that are truncated in the D3-D4 loop and in the D1-D2 loop respectively (Fig 1A). A missense mutation, S1231T, which results in an amino acid substitution in the D3S1 segment, was observed in Patient 3 (Fig 1A). A mutation in an adjacent nucleotide of the same codon is responsible for the missense mutation S1231R in a Japanese patient with severe myoclonic epilepsy of infancy [10].

In all families, parental identity was confirmed by analysis of microsatellite markers from multiple chromosomes. In agreement with previous findings, these mutations were not observed in the parents, indicating that they arose *de novo* in the patients. Although the family history of Patient 2 includes absence seizures in the mother and one febrile seizure in the father, neither parent carries the *SCN1A* mutation.

## Discussion

Five of the six mutations described in this paper have not previously been reported. R613X, P707fsX715, and S914fsX934 truncate >50% of the Nav1.1 protein, making it unlikely to generate a functional channel [11]. The mutation R1525X in the D3-D4 loop truncates the final 485 of the total of 2009 amino acids, and K1846fsX1856 removes the final 153 residues of the C-terminus (Fig 1A). Biophysical characterization of two similarly truncated Nav1.1 mutants, R712X and R1892X, demonstrated that both lacked channel activity [11]. Truncation of the cardiac sodium channel *SCN5A* at nearby positions also destroys channel activity

[12]. With regard to missense mutations, both loss-of-function alleles and gain-of-function alleles with noninactivating channel activity have been observed [6,13]. Biophysical analysis will be required to determine the effect of S1231T on channel function.

*De novo* mutations in *SCN1A* are responsible for approximately 50% of the incidence of severe myoclonic epilepsy of infancy. At least 150 independent mutations have been identified in affected children, and more than 90% are sporadic rather than inherited [2]. Approximately half of the severe myoclonic epilepsy of infancy mutations result in premature truncation of the channel protein and loss of channel activity. Clinical severity is comparable for truncations close to the N-terminus of the protein and those close to the C-terminus, demonstrating haploinsufficiency for *SCN1A*. Many of the missense mutations in severe myoclonic epilepsy of infancy also result in loss of function [6].

Haploinsufficiency for *SCN1A* could lead to reduced sodium currents in inhibitory interneurons resulting in hyperexcitability. Mice with only 50% of normal Nav1.1 due to heterozygosity for a targeted null allele of *Scn1a* were found to have spontaneous seizures and reduced lifespan [14]. Recordings of hippocampal neurons from these mice demonstrate reduced sodium current density in inhibitory interneurons but not in excitatory pyramidal neurons, supporting the hypothesis that reduced inhibitory input is responsible for hyperexcitability.

## Review of Previously Described *SCN1A* Mutations in Severe Myoclonic Epilepsy of Infancy

The *de novo* mutation K1846fsX1856, identified in Patient 2, has been previously identified as a *de novo* mutation in two unrelated patients with severe myoclonic epilepsy of infancy [4,15]. The major clinical features of

**Table 2. Fifteen recurrent mutations in unrelated patients with SMEI.**

Mutation	Mechanism	Nucleotide Change	CpG Codon	Exon	Cases (n)
K1846fsX1856	Slipped-strand mispairing	ΔAAAAC	n.a.	26	3
L1670fsX1678	Slipped-strand mispairing	ΔGTTT	n.a.	26	2
R222X	<sup>m</sup> CpG deamination	C > T	<u>C</u> GA	5	4
R712X	<sup>m</sup> CpG deamination	C > T	<u>C</u> GA	12	4
R1245X	<sup>m</sup> CpG deamination	C > T	<u>C</u> GA	19	2
R1407X	<sup>m</sup> CpG deamination	C > T	<u>C</u> GA	21	2
R1892X	<sup>m</sup> CpG deamination	C > T	<u>C</u> GA	26	3
R946C	<sup>m</sup> CpG deamination	C > T	<u>C</u> GC GTG	15	2
R931C	Transition	C > T	n.a.	15	2
W952X	Transition	G > A	n.a.	15	2
Splice site	Transition	G > A	n.a.	22+1	2
W1434R	Transition	T > C	n.a.	22	2
S1516X	Transversion	C > A	n.a.	24	2
I227S	Transversion	T > G	n.a.	5	3
M934I	Transversion	G > C	n.a.	15	2

Most cases arose de novo. For primary references see [3,4]. Amino acid residues correspond to the *SCN1A* cDNA isoform containing the long form of exon 10 (Genbank Accession AB093548).

Abbreviations:

n.a. = Not applicable

SMEI = Severe myoclonic epilepsy of infancy

these three affected individuals include early onset (between 2 and 5 months of age) with generalized tonic-clonic seizures and myoclonic seizures. All three patients exhibited severe mental retardation, and one of the cases from the literature was also ataxic. Reduced sodium channel activity thus appears to affect multiple brain regions. The tetranucleotide that is deleted in these patients is part of a direct repeat, AAACAAAC, in exon 26 (Fig 1B). Slipped-strand mispairing during DNA replication is a mechanism that could account for the recurrent mutations at this site.

The recurrence of K1846fsX1856 prompted us to review the frequency of reoccurrence of previously described *SCN1A* mutations in severe myoclonic epilepsy of infancy. Published reports include 15 recurrent mutations (Table 2). Both of the recurrent deletions affect short direct repeats that are susceptible to slipped-strand mispairing (Table 2). Six recurrent C to T transitions occur at CpG dinucleotides, known to be susceptible to deamination of methylated cytosine. Five of these change the arginine codon CGA to the nonsense codon TGA. CGA is the only codon for which deamination of a CpG dinucleotide generates a stop codon. To date, 122 different mutations in *SCN1A* have been identified in 144 patients with severe myoclonic epilepsy of infancy [4,5]. The 15 recurrent mutations were observed in 37 patients, and thus account for 25% of *SCN1A*-associated severe myoclonic epilepsy of infancy (37/144). An efficient mutation screening strategy would begin with the eight exons in which these recurrent mutations are present. However, the majority of *SCN1A* mutations in patients with severe myoclonic epilepsy of infancy are private mutations.

While definitive identification of a *SCN1A* mutation might allow earlier confirmation of the diagnosis of

severe myoclonic epilepsy of infancy, and selection of therapies with greater efficacy in this condition, there is currently no correlation between *SCN1A* mutations and response to specific therapies. Our ability to personalize treatment of this disorder is likely to increase in the future, as the molecular lesion is identified in more patients, and additional therapeutic agents are developed.

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