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De Novo Mutations in the Sodium-Channel Gene *SCN1A* Cause Severe Myoclonic Epilepsy of Infancy

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Severe myoclonic epilepsy of infancy (SMEI) is a rare disorder that occurs in isolated patients. The disease is characterized by generalized tonic, clonic, and tonic-clonic seizures that are initially induced by fever and begin during the first year of life. Later, patients also manifest other seizure types, including absence, myoclonic, andsimple and complex partial seizures. Psychomotor development stagnates around the second year of life. Missense mutations in the gene that codes for a neuronal voltage-gated sodium-channel α-subunit (*SCN1A*) were identified in families with generalized epilepsy with febrile seizures plus (GEFS+). GEFS+ is a mild type of epilepsy associated with febrile and afebrile seizures. Because both GEFS+ and SMEI involve fever-associated seizures, we screened seven unrelated patients with SMEI for mutations in *SCN1A*. We identified a mutation in each patient: four had frameshift mutations, one had a nonsense mutation, one had a splice-donor mutation, and one had a missense mutation. All mutations are de novo mutations and were not observed in 184 control chromosomes.

Introduction

Severe myoclonic epilepsy of infancy (SMEI) was first described by Dravet in 1978 and is included as an epileptic syndrome in the International League Against Epilepsy (ILAE) classification, which was proposed by the Commission on Classification and Terminology of the ILAE (1989). Early manifestations of the disease are tonic, clonic, and tonic-clonic seizures that occur within the first year of life. These seizures are often prolonged, generalized, and associated with fever. Later in life, patients with SMEI have afebrile seizures, including myoclonic, tonic-clonic, absence, and simple and complex partial seizures. Early psychomotor and speech development is normal, but developmental stagnation occurs during the second year of life. Patients often become ataxic, and speech development is delayed. In general, SMEI is very resistant to all forms of pharmacotherapy.

A mild phenotype characterized by febrile seizures and, occasionally, epilepsy in adulthood was described by Scheffer and Berkovic (1997) as "generalized epilepsy with febrile seizures plus" (GEFS+) (MIM 604233). Two families with GEFS+ were reported to segregate missense mutations in the gene that codes for the α-subunit of a neuronal voltage-gated sodium channel (*SCN1A*) located on chromosome 2q (Escayg et al. 2000). Both mutations are within the transmembrane segments DII S4 and DIV S4 (fig. 1), which are voltage sensors of the channel. Recently, additional families with GEFS+ were reported to have novel missense mutations in *SCN1A* (Escayg et al. 2001; Wallace et al. 2001), which were located in the linker between DII and DIII and in the DI S2-S3 loop, DIII S5, and DIV S4 (fig. 1).

Because fever-induced seizures occur both in GEFS+ and SMEI, we screened *SCN1A* for mutations in seven patients with SMEI.

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Subjects and Methods

Subjects

We studied seven Belgian patients with SMEI, diagnosed according to the criteria of the Commission on Classification and Terminology of the ILAE (1989). DNA was extracted from peripheral blood of the seven patients, their unaffected parents, and 92 healthy control persons randomly selected from the Belgian population. The study was approved by the Commission for Medical Ethics of the University of Antwerp.

Human SCN1A

We defined the exon-intron boundaries of *SCN1A* by aligning the genomic sequence contained in BAC clone

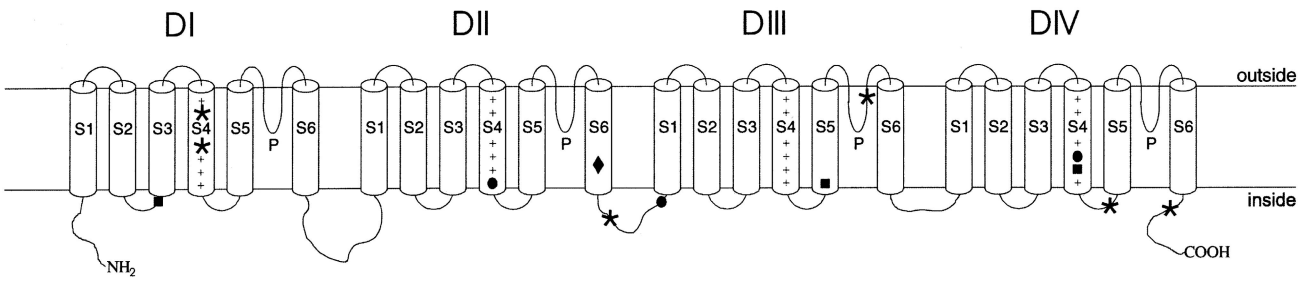

Figure 1 Organization of SCN1A. The neuronal voltage-gated sodium-channel α-subunit SCN1A is a monomer and consists of four homologous domains (DI–DIV). Each domain has six transmembrane segments (S1–S6). S4 has several positively charged amino acids and represents the voltage sensor. P = the pore loop, which delineates the pore of the channel. Mutations identified in this study (described in table 2) were denoted as follows: asterisks (*) = deletion, insertion and nonsense mutations; diamond (♦) = missense mutation; circles (●) = GEFS+ missense mutations reported by Escayg et al. (2000, 2001); and squares (■) = GEFS+ missense mutations reported by Wallace et al (2001).

RP11-2I8 (Genbank accession number AC010127), the cDNA sequence of *SCN8A* (Genbank accession number XM_006838), and the protein sequence of *SCN1A* (Escayg et al. 2000). Recently, the complete cDNA and protein sequence of *SCN1A* was submitted to Genbank (accession numbers AF225985 and AAK00217). This sequence represents an alternatively spliced form of *SCN1A* that was described by Schaller et al. (1992). The protein sequence published by Escayg et al. (2000) contains an additional 11 amino acids at the 3′ end of exon 11, which encode part of the cytoplasmatic loop between DI and DII.

Mutation Detection and Molecular-Genetic Analysis

Intronic primer pairs were designed for each of the 26 exons, on the basis of the genomic sequence, using

Table 1

Sequence of the Intronic Primers (5′→3′) Used for the Mutation Analysis of *SCN1A*

Exon	5′ Primer (Forward)	3′ Primer (Reverse)	No. of Base Pairs in PCR Product
1	TCATGGCACAGTTCCTGTATC	GCAGTAGGCAATTAGCAGCAA	589
2	TGGGGCACTTTAGAAATTGTG	TGACAAAGATGCAAAATGAGAG	391
3	GCAGTTTGGGCTTTTCAATG	TGAGCATTGTCCTCTTGCTG	314
4	AGGGCTACGTTTCATTTGTATG	TGTGCTAAATTGGAATCCAGAG	421
5	CAGCTCTTCGCACTTTCAGA	TCAAGCAGAGAAGGATGCTGA	307
6	AGCGTTGCAAACATTCTTGG	GGGATATCCAGCCCCTCAAG	477
7	GACAAATACTTGTGCCTTTGAATG	ACATAATCTCATACTTTATCAAAAACC	362
8	GAAATGGAGGTGTTGAAAATGC	AATCCTTGGCATCACTCTGC	581
9	AGTACAGGGTGCTATGACCAAC	TCCTCATACAACCACCTGCTC	440
10	TCTCCAAAAGCCTTCATTAGG	TTCTAATTCTCCCCCTCTCTCC	544
11	TCCTCATTCTTTAATCCCAAGG	GCCGTTCTGTAGAAACACTGG	670
12	GTCAGAAATATCTGCCATCACC	GAATGCACTATTCCCAACTCAC	372
13	TGGGCTCTATGTGTGTGTCTG	GGAAGCATGAAGGATGGTTG	543
14	TACTTCGCGTTTCCACAAGG	GCTATGCAGAACCCTGATTG	433
15	ATGAGCCTGAGACGGTTAGG	ATACATGTGCCATGCTGGTG	544
16	TGCTGTGGTGTTTCCTTCTC	TGTATTCATACCTTCCCACACC	658
17	AAAAGGGTTAGCACAGACAATG	ATTGGGCAGATATAATCAAAGC	489
18	CACACAGCTGATGAATGTGC	TGAAGGGCTACACTTTCTGG	567
19	TCTGCCCTCCTATTCCAATG	GCCCTTGTCTTCCAGAAATG	445
20	AAAAATTACATCCTTTACATCAAACTG	TTTTGCATGCATAGATTTTCC	395
21	TGAACCTTGCTTTTACATATCC	ACCCATCTGGGCTCATAAAC	579
22	TGTCTTGGTCCAAAATCTGTG	TTGGTCGTTTATGCTTTATTCG	283
23	CCCTAAAGGCCAATTTCAGG	ATTTGGCAGAGAAAACACTCC	378
24	GAGATTTGGGGGTGTTTGTC	GGATTGTAATGGGGTGCTTC	600
25	CAAAAATCAGGGCCAATGAC	TGATTGCTGGGATGATCTTG	483
26a	AGGACTCTGAACCTTACCTTGG	TGTACATGTTCACCACAACCAG	589
26b	TGTGGGAACCCATCTGTTG	CCATGAATCGCTCTTCCATC	418
26c	TGCTTTTACAAAGCGGGTTC	GTTTGCTGACAAGGGGTCAC	592

Table 2

***SCN1A* Mutations in Patients with SMEI**

Patient Number	Location in *SCN1A*	DNA Change[a]	Position in *SCN1A*	Mutation	Protein Change
EP 153	Exon 5	c.657-658delAG	DI S4	Frameshift, premature stop codon	S219fsX275
EP 78	Exon 5	c.664C→T	DI S4	Premature stop codon	R222X
EP 147	Exon 16	c.2956C→T	DII S6	Missense mutation	L986F
EP 91	Exon 16	c.3299-3300insAA	DII-DIII linker	Frameshift, premature stop codon	K1100fsX1107
EP 64	Intron 22	IVS22+1G→A	DIII S5-S6 pore	Splice donor	–
EP 90	Exon 26	c.5010-5013delGTTT	DIV S4-S5 loop	Frameshift, premature stop codon	L1670fsX1678
EP 89	Exon 26	c.5536-5539delAAAC	C-terminal	Frameshift, premature stop codon	S1846fsX1856

[a] Numbering of mutations started from the initiating ATG codon, as described by Escayg et al. (2000).

the Primer3 program (table 1). Exons and splice-site junctions were amplified using PCR, from 10 ng genomic DNA in a standard PCR procedure that used Platinum *Taq* (Life Technologies). Next, PCR fragments were denatured at 95°C for 4 min, were slowly cooled to room temperature, and subsequently were analyzed by denaturing high performance liquid chromatography (DHPLC) on the WAVE automated instrument (Transgenomic), as described elsewhere (Underhill et al. 1997). Each fragment with an aberrant DHPLC pattern was purified using the PCR product presequencing kit (USB). Subsequently, the products were sequenced using the BigDye Terminator Cycle Sequencing kit (PE Applied Biosystems) and forward and reverse primers. Sequence reactions were performed on an ABI 3700 automated sequencer (PE Applied Biosystems). Data were collected and analyzed using the ABI DNA sequence analysis software, version 3.6.

We used pyrosequencing, as described by Alderborn et al. (2000), or PCR-RFLP analysis to screen the control population for the presence of the *SCN1A* mutations observed in the patients. The following primers were used for pyrosequencing: 5′-AAATCGTCTTCAATGCTCGG-3′ (c.657-658delAG), 5′-GAAATCGTCTTCAATG-3′ (c.664C→T), 5′-AGAAGCAAGGCCAGAAAG-3′ (c.2956C→T), 5′-GTTGAAAAATACATTATTG-3′ (c.3299-3300insAA), 5′-TGTATGCAGCAGTTGATTCC-3′ (IVS22+1G→A), and 5′-CAATCTGCCACAACCAAA-3′ (c.5536-5539delAAAC). The restriction enzyme *Hin*cII was used for the PCR-RFLP, thereby cutting the wild-type 589-bp fragment of exon 26a into fragments of 483 and 106 bp. The c.5010-5013delGTTT mutation creates an extra *Hin*cII restriction site in exon 26a, and digestion of the fragment results in three fragments, of 254, 225, and 106 bp.

The *SCN1A* mutations were described according to the nomenclature established by den Dunnen and Antonarakis (2000), and numbering was started from the initiating ATG codon, as described by Escayg et al. (2000).

Paternity Testing

We genotyped D21S188, a multiallelic microsatellite marker on chromosome 21p (Wang et al. 1999). This marker shows an average of 16 distinct alleles derived from eight chromosomes. In addition, we genotyped several highly informative microsatellite markers on chromosomes 17 (D17S785, D17S802, and D17S1847) and 18 (D18S51, D18S68, and D18S465). Heterozygosity frequencies of the chromosome 17 and 18 markers are 74%–89% (CEPH genotype database).

Results

SCN1A *Mutation Analysis*

All 26 exons and exon-intron boundaries of *SCN1A* were analyzed for mutations in seven patients with SMEI by DHPLC analysis of PCR-amplified fragments (table 1). In each patient with SMEI, we observed a single aberrant *SCN1A* fragment (table 2). Subsequent sequence analysis identified a heterozygous mutation in each patient: three out-of-frame deletions; one insertion; and one nonsense, one splice-donor, and one missense mutation (fig. 2). Table 2 summarizes the mutation data, and figure 1 illustrates the location of the mutations within the SCN1A protein. The deletions and insertion result in a frameshift with a premature stop codon: c.657-658delAG, c.3299-3300insAA, c.5010-5013delGTTT, and c.5536-5539delAAAC. The 5′ splice-site mutation (IVS22+1G→A) most likely leads to either exon skipping or use of a cryptic splice-donor site. A potential splice-donor consensus GT starts at IVS22+45, which, if used, results in a premature stop codon in exon 23. The missense mutation c.2956C→T results in a change from leucine to phenylalanine amino acid. The leucine at codon 986 is highly conserved among paralogical and orthological sodium-channel α-subunits (fig. 3). Most likely the L986F causes severe damage to *SCN1A*.

We used sequencing analysis to determine whether any patient's mutation was present in either of the unaffected parents. In all cases, the mutation was absent from the

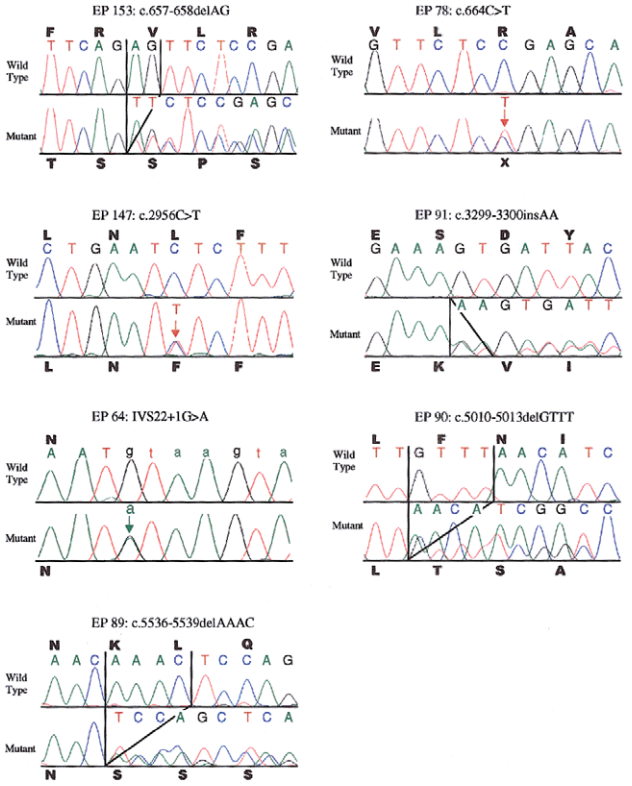

Figure 2 Chromatograms of the *SCN1A* mutations in comparison with *SCN1A* wild-type sequences. Exonic sequences are in uppercase; intronic sequences are in lowercase.

parents. Also, for all patients, paternity was confirmed using several microsatellite markers. For each mutation (except for the c.5010-5013delGTTT mutation), we developed a pyrosequencing assay and showed that the mutations were absent from 92 control individuals. The c.5010-5013delGTTT mutation creates a new *Hin*cII restriction site, and we used PCR-RFLP to show that the mutation was absent in 92 control individuals (data not shown).

Phenotype of Patients with SMEI

Among the seven patients with SMEI, the age at onset of disease was 2–6 mo (table 3). In all patients, the earliest seizures were generalized; in four of the seven patients, these seizures were associated with fever. Subsequent seizures included secondary generalized tonic-clonic, myoclonic, absence, and simple and complex partial seizures. In all patients, the seizures were resistant to therapy. All patients became mentally retarded, and five patients have ataxia. One patient died at the age of 4 years.

Discussion

In seven patients with SMEI, we identified a heterozygous mutation in *SCN1A* that was absent in their parents, thereby providing substantial evidence that (1) SMEI has a genetic etiology and (2) de novo mutations in *SCN1A* are probably a major cause of SMEI. Heterozygous *SCN1A* mutations have also been reported in families with autosomal dominant GEFS+. However, all GEFS+ mutations are missense mutations that display reduced penetrance. In contrast, all cases of SMEI occur in isolated patients. The difference in phenotype and severity is reflected in the nature of *SCN1A* mutations identified in patients with SMEI. In five of seven patients with SMEI, we identified frameshift deletions (c.657-658delAG, c.5010-5013delGTTT, c.5536-5539delAAAC) or an insertion (c.3299-3300insAA) or a nonsense mutation (c.664C→T). Also, in one patient with SMEI, we identified a G→A transition in the conserved GT consensus sequence of the splice-donor site of intron 22. The consequence of this mutation can only be elucidated by analyzing the mutated transcript or protein. In one patient with SMEI, we identified a missense mutation at codon 986 (L986F), which is located within domain DII S6 of the SCN1A protein. Since codon 986 is highly conserved among α-subunits of human and other species, it can be predicted that this mutation may have a deleterious effect on SCN1A.

Thus, in the majority of patients with SMEI, the mutation results in early termination of translation, thereby producing a C-truncated SCN1A protein from one of the *SCN1A* alleles. Rapid degradation of these truncated transcripts or proteins could lead to a loss of function comparable with haploinsufficiency. Alternatively, some of the transcripts could lead to abnormal proteins

	IIS6	Accession no.
Human SCN1A	MCLTVFMMVMVIGNLVVLNLFLALLL	AAK00217
Rat scn1a	.....R.....	AAA79965
Human SCN2A	.....	NP_066287
Human SCN3A	...I...L.....	AAK00219
Rat scn3a	...I...L.....	NP_037251
Human SCN4A	......L.....	NP_000325
Human SCN5A	L..L..LL.....	NP_000326
Human SCN6A	W.IPFYL..IL....L..Y.....V-	NP_002967
Human SCN8A	...I.....	XP_006838
Human SCN9A	...I.Y.....	NP_002968
Human SCN10A	I..IL.LT...L.....I....	NP_006505
Human SCN11A	L.VI..ILIT...K.....I....	AAF17480
Human SCN12A	L.VI..ILIT...K.....I....	NP_054858
Fugu	...I.....	BAA07195
Squid	-.VPF.LLT.I.....	AAA16202
Electric Eel	...A.Y...II.....M.....	CAA25587
Drosophila	S.IPF.LATV.....	AAB59192

Figure 3 Evolutionary conservation of *SCN1A*. Sequence alignment and evolutionary conservation of the 986L residue (boxed).

Table 3

Clinical Features of Seven Patients with SMEI with Mutations in *SCN1A*

Patient Characteristic	Data for Patients						
	EP 64	EP 78	EP 89	EP 90	EP 91	EP 147	EP153
Sex	Male	Male	Male	Female	Female	Female	Male
Year of birth	1994	1994	1988	1995	1992	1998	1990
Race	White	White	White	White	White	White	White
Status at birth	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Family history:							
Consanguinity	−	−	−	−	−	−	−
Febrile seizures	−	Paternal uncle	−	Mother	−	−	Maternal niece
Epilepsy	−	−	−	−	Maternal niece, paternal uncle	−	−
First seizure:							
Age	5 mo	6 mo	2 mo	4 mo	4 mo	4 mo	3 mo
Type	GTCS	GCS	GTS	GTCS	GCS	GTCS	GTCS
Fever-associated	+	−	−	−	+	+	+
Other seizures:							
Secondary GTCS	+	+	+	+	+	+	+
Myoclonic seizures:	+	+	+	+	+	+	+
Age at onset	>7 mo	>10 mo	>3 mo	>12 mo	>8 mo	>12 mo	>3 years
Frequency	Daily	Daily	Daily	Rarely	Daily	Rarely	Rarely
Other seizures	CPS	Abs	−	Abs	−	Abs, SPS	Abs
Other features:							
Therapy-resistant	+	+	+	+	+	+	+
Mental retardation	Moderate	Moderate	Severe	Severe	Severe	Moderate	Moderate
Ataxia	±	+	+	−	−	+	+
Age at last examination	6 years	6 years	10 years	5 years	6 years	2 years	4 years[a]

Note.—− = absent; + = present; ± = probably present; GTCS = generalized tonic-clonic seizures; GCS = generalized clonic seizures; GTS = generalized tonic seizures; Abs = absence seizures; SPS = simple partial seizures; CPS = complex partial seizures.

[a] Died at age 4 years.

with a toxic increase in function. In this respect, it is of intrest that heterozygous knockout mice for three voltage-gated sodium-channel α-subunits (*SCN2A*$^{+/-}$, *SCN8A*$^{+/-}$, and *SCN10A*$^{+/-}$) do not display an abnormal phenotype and appear to develop normally. In contrast, homozygous knockout mice for these genes have a severe phenotype. *SCN2A*$^{-/-}$ mice die, as a result of severe hypoxia and extensive neuronal cell death, within 1–2 d of birth (Planells-Cases et al. 2000). *SCN8A*$^{-/-}$ mice display a paralytic phenotype, with muscle atrophy and ataxia, and die ≤4 wk after birth (Burgess et al. 1995). *SCN10A* is a sensory-neuron–specific voltage-gated sodium channel. *SCN10A*$^{-/-}$ mice have partial deficits in perception of noxious thermal, mechanical, and inflammatory stimuli (Akopian et al. 1999). Given these observations, we conclude that the analysis of mutant transcripts and proteins is necessary for elucidation of the exact pathogenetic mechanism of heterozygous *SCN1A* mutations in patients with SMEI.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Genbank, http://www.ncbi.nlm.nih.gov/Genbank (for genomic clone containing *SCN1A* [accession number AC010127], human *SCN1A* [for cDNA accession number AF225985, for protein accession number AAK00217], *Rattus norvegicus Scn1a* [accession number AAA79965], human *SCN2A* [accession number NP_066287], human *SCN3A* [accession number AAK00219], *Rattus norvegicus Scn3a* [accession number NP_037251], human *SCN4A* [accession number NP_000325], human *SCN5A* [accession number NP_000326], human *SCN6A* [accession number NP_002967], human *SCN8A* [for cDNA accession number XM_006838, for protein accession number XP_006838], human *SCN9A* [accession number NP_002968], human *SCN10A* [accession number NP_006505], human *SCN11A* [accession number AAF17480], human *SCN12A* [accession number NP_054858], *Takifugu rubripes* [accession number

BAA07195], *Loligo opalescens* [accession number AAA16202], *Electrophorus electricus* [accession number CAA25587], *Drosophila melanogaster* [accession number AAB59192])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for GEFS+ [MIM 604233])

Primer3 program, http://www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi

CEPH genotype database, http://www.cephb.fr/cephdb/dumps.html

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