

RESEARCH ARTICLE

De Novo *SCN1A* Mutations Are a Major Cause of Severe Myoclonic Epilepsy of Infancy

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Severe myoclonic epilepsy of infancy (SMEI or Dravet syndrome) is a rare disorder occurring in young children often without a family history of a similar disorder. The earliest disease manifestations are usually fever-associated seizures. Later in life, patients display different types of afebrile seizures including myoclonic seizures. Arrest of psychomotor development occurs in the second year of life and most patients become ataxic. Patients are resistant to antiepileptic drug therapy. Recently, we described de novo mutations of the neuronal sodium channel α -subunit gene *SCN1A* in seven isolated SMEI patients. To investigate the contribution of *SCN1A* mutations to the etiology of SMEI, we examined nine additional SMEI patients. We observed eight coding and one noncoding mutation. In contrast to our previous study, most mutations are missense mutations clustering in the S4-S6 region of *SCN1A*. These findings demonstrate that de novo mutations in *SCN1A* are a major cause of isolated SMEI. *Hum Mutat* 21:615–621, 2003. © 2003 Wiley-Liss, Inc.

KEY WORDS: *SCN1A*; severe myoclonic epilepsy of infancy; SMEI; Dravet syndrome; generalized epilepsy with febrile seizures plus; GEFS+; epilepsy

DATABASES:

SCN1A – OMIM: 182389 (*SCN1A*), 604233 (GEFS+), 607208 (SMEI)

INTRODUCTION

Epilepsy is a common neurologic disorder that affects more than 3% of the population at some time during life. A genetic contribution has been suggested for about 40% of epilepsy patients. In recent years, progress has been made in understanding the genetic etiology of epilepsies. Ion channel mutations have been reported in several families with distinct epilepsy phenotypes: ADNFLE (autosomal dominant nocturnal frontal lobe epilepsy) is associated with mutations in *CHRNA4* and *CHRNA2* [Steinlein et al., 1995; De Fusco et al., 2000], while mutations in *KCNQ2* and *KCNQ3* lead to BFNC (benign familial neonatal convulsions) [Singh et al., 1998; Charlier et al., 1998; Biervert et al., 1998]. In generalized epilepsy with febrile seizures plus (GEFS+; MIM# 604233), mutations have been reported in *SCN1B* [Wallace

et al., 1998], *SCN1A* [MIM# 182389; Escayg et al., 2000, 2001; Wallace et al., 2001a; Sugawara et al., 2001a; Abou-Khalil et al., 2001], *SCN2A* [Sugawara

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et al., 2001b], and *GABRG2* [Baulac et al., 2001; Wallace et al., 2001b; Harkin et al., 2002; Marini et al., 2003]. More recently, de novo mutations in *SCN1A* in patients with severe myoclonic epilepsy of infancy (SMEI or Dravet syndrome; MIM# 607208) were described [Claes et al., 2001; Sugawara et al., 2002; Ohmori et al., 2002]. Finally, mutations in *LGII* were shown to cause partial epilepsy with auditory features [Kalachikov et al., 2002; Morante-Redolat et al., 2002; Gu et al., 2002], while mutations in *GABRA1* cause autosomal dominant juvenile myoclonic epilepsy [Cossette et al., 2002].

SMEI is a rare disorder recognized as an epilepsy syndrome by the International League Against Epilepsy (ILAE). In 2001, the classification of epilepsy syndromes was revised, and SMEI was classified among the epileptic encephalopathies as "Dravet syndrome" [Engel, 2001]. Dravet was the first to describe patients with this syndrome in 1978 [Dravet, 1978]. The frequency of SMEI was estimated at 1 in 40,000 children and represented 7% of all severe epilepsies starting before the age of 3 years. Infants with this disorder develop normally during the first year of life. However, initial seizures already occur between 2 and 9 months after birth. These seizures are prolonged tonic, clonic, or tonic-clonic seizures. These are usually generalized and associated with fever. In the second year of life, seizures are no longer fever-induced and include myoclonic, tonic-clonic, absence, and partial seizures. Psychomotor and speech development delays and eventually comes to a halt. Often, patients also develop ataxia. SMEI is an intractable epilepsy; seizures are often difficult to control and there is no treatment that fundamentally alters the outcome.

Recently, we described de novo mutations in *SCN1A* in seven isolated patients diagnosed with the SMEI phenotype [Claes et al., 2001]. Here, we describe the results of the mutation analysis of *SCN1A* for nine additional SMEI patients. We identified nine mutations, of which eight were novel. All mutations occurred de novo. In contrast to our previous findings, where we identified mainly nonsense and frameshift mutations, we now identified mostly missense mutations located in the region S4-S6 of *SCN1A*. These findings emphasize the importance of *SCN1A* mutations in the genetic etiology of SMEI.

SUBJECTS AND METHODS

Subjects

We ascertained nine isolated patients with SMEI. As there are no strict inclusion or exclusion criteria, these patients were diagnosed as SMEI because their clinical features perfectly correspond to the phenotype described in the ILAE classification under SMEI or Dravet syndrome [Engel, 2001]. DNA of the patients and the parents was extracted from peripheral blood. DNA was also obtained from 92 healthy control individuals randomly selected from the population. All

participants or their parents signed an informed consent for participation in our study. The Medical Ethical Committee of the University of Antwerp approved our study.

Mutation Analysis

For the mutation analysis of the 26 *SCN1A* exons we used genomic DNA, and PCR amplification was performed using flanking intronic primers as described previously [Claes et al., 2001]. Amplification occurred in a standard PCR reaction using HotGoldStar Taq (Eurogentech, Seraing, Belgium). To facilitate the formation of heteroduplexes for denaturing high performance liquid chromatography (DHPLC) analysis (WAVE, Transgenomic, Omaha, NE) [Underhill et al., 1997], PCR products were denatured at 95°C for 4 min and slowly cooled to room temperature. PCR fragments showing altered DHPLC patterns were sequenced on an ABI 3700 automated sequencer (PE Applied Biosystems, Foster City, CA).

Pyrosequencing [Alderborn et al., 2000] was used to screen the control sample for the observed mutations. The following SNP primers were used for pyrosequencing: 5'-TGGTGGTACAAGCACTG-3' (c.7C>T), 5'-TTTTCC-CAGCAGCA-3' (c.1178G>A), 5'-GGAAGAAGTCATTC-ATGTG-3' (c.2796G>A), 5'-CGGAACACAATCAGGA-3' (c.2817C>G), 5'-CCAGCAACCTCCATAC-3' (c.2875T>C), 5'-GGCCACATTCAAAGG-3' (c.4300T>C), 5'-TGTATGC-AGCAGTTGATTCC-3' (IVS22+1G>A), 5'-CGCACGCT-GCTCT-3' (c.4982T>C), and 5'-TCTCCCTTAACCTGAG-CTT-3' (c.5246G>A).

The positions of the mutations were numbered from the start codon ATG [Escayg et al., 2000], and mutation nomenclature definitions described by den Dunnen and Antonarakis [2001] were used to describe the mutations.

Paternity Testing

To test paternity, we genotyped the multiallelic microsatellite marker D21S188 [Wang et al., 1999] by PCR amplification of genomic DNA and analyzed the alleles on the ABI 3700 sequencer using Genotyper[®] 3.6 (Applied Biosystems). This chromosome 21p (CA)_n dinucleotide repeat marker shows, on average, 16 different alleles derived from eight different chromosomes.

RESULTS

Clinical Features of SMEI Patients

Of the nine patients included in our study, eight were female and one was male. One patient had Jewish Ashkenazi ethnicity; the other eight patients were Caucasians. The mean age of the patients at the time of DNA analysis was 9 years, ranging from 1.9 to 25 years, and their status at birth was normal. No consanguinity was present among the parents.

An overview of the clinical features of the nine patients is given in Table 1. The disease onset ranged from 2.5 to 9 months. In six of nine patients, the first seizures were associated with fever. Subsequent seizures were generalized tonic-clonic seizures in eight patients, myoclonic seizures in eight patients, absence seizures in four patients, and complex partial seizures in four patients. Status epilepticus is mentioned in at least four patients. All patients were therapy-resistant and showed delayed mental development

TABLE 1. Clinical Features of the Nine SMEI Patients With Mutations in SCN1A

	EP 152	EP 158	EP 159	EP 164	EP 173	EP 181	EP 185	EP 192	EP 193
Status at birth	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Family history									
consanguinity	-	-	-	-	-	-	-	-	-
febrile seizures	-	-	-	-	-	-	-	-	-
epilepsy	Maternal niece	Post traum. ep	-	Maternal grandmother	-	-	-	First cousin	-
First seizure									
age	6 months	3 months	7 months	8 months	9 months	6 months	4.5 months	7 months	2.5 months
type	MS	LTCS	GTCS	GTCS	SE	GTCS	LTCS	GTCS	MS
fever-associated	-	+	+	+	+	+	-	+	?
other features	-	-	-	-	-	-	After 1st vaccination	-	-
Other seizures									
secondary GTCS	+	+	+	+	+	+	+	GCS	+
myoclonic seizures:	+	+	+	+	+	+	-	+	+
onset	6 months	10 months	3 months	2 years	15 months	2 years	-	8 months	2 months
frequency	Daily	Weekly	Daily	Weekly	Daily	Weekly	-	Monthly	Daily
other seizures	abs, CPS, SE	abs, SE	-	abs, CPS	LTCS	CPS	SE, GTCS, GTS, CPS	GTCS, LCTS	abs
Other features									
therapy resistant	+	+	+	+	+	+	+	+	+
mental retardation	Severe	Severe	Severe	Moderate	Severe	Moderate	Moderate	?	Moderate
ataxia	+	+	+	+	-	+	+	-	+
last examination at	7.5 years	16 years	25 years	12.5 years	6.5 years	5.5 years	1.9 years	3.5 years	2 years

- absent; +, present; ?, unknown; GTCS, generalized tonic-clonic seizures; LTCS, lateralized tonic-clonic seizures; GCS, generalized clonic seizures; GTS, generalized tonic seizures; abs, absence seizures; SE, status epilepticus; MS, myoclonic seizures; CPS, complex partial seizures.

TABLE 2. SCN1A Mutations in SMEI Patients

Patient	Location in SCN1A	DNA change	Protein change	Mutation	Position in SCN1A
EP 192	Exon 1	c.7C>T	Q3X	Premature stop codon	N-terminal
EP 152	Exon 9	c.1178G>A	R393H	Missense	DIS5-S6
EP 159	Exon 15	c.2796G>A	W932X	Premature stop codon	DIIS5-S6
EP 185	Exon 15	c.2817C>G	H939Q	Missense	DIIS5-S6
EP 158	Exon 15	c.2875T>C	C959R	Missense	DIIS5-S6
EP 193	Exon 22	c.4300T>C	W1434R	Missense	DIIS5-S6
EP 181	Intron 22	IVS22+1G>A	-	Splice donor	DIIS5-S6
EP 173	Exon 26	c.4982T>C	F1661S	Missense	DIVS4-S5
EP 164	Exon 26	c.5246G>A	G1749E	Missense	DIVS5-S6

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

that eventually arrested. Seven patients showed signs of ataxia. Sometimes the complete phenotype had not yet fully developed because of the young age of the patients.

Mutation Analysis of SCN1A

Amplification of the 26 exons of SCN1A and subsequent analysis by DHPLC resulted in a single aberrant DHPLC pattern for each of the nine SMEI patients. Sequencing revealed a heterozygous mutation in each patient. We identified six missense, two nonsense, and one splice donor site mutation (Table 2). Sequence analysis of the parents' DNA revealed that none of the parents carried the mutation, indicating that all mutations were de novo mutations. We confirmed paternity for all patients. Using pyrosequencing, we demonstrated that the

mutations were absent from 184 control chromosomes. In summary, we found nine de novo mutations in SCN1A, of which eight were novel.

We also identified several novel intronic and exonic sequence variants not yet present in dbSNP (www.ncbi.nlm.nih.gov/SNP/). The allele frequencies calculated in the 16 SMEI patients are summarized in Table 3. Exonic variants were silent mutations.

DISCUSSION

Previously, we reported seven patients with SMEI who carried a heterozygous de novo mutation in SCN1A. Six out of seven patients had a nonsense or splice-site mutation, suggesting SMEI is caused by a loss of function comparable to haploinsufficiency. To further test the contribution of SCN1A mutations in the genetic etiology of SMEI, we performed

a mutation analysis in nine additional SMEI patients. We identified a heterozygous mutation in each patient that was absent from the parents and 92 control individuals. Six missense, two nonsense, and one splice donor site mutation were identified. All mutations, except for the splice donor site mutation located in the DIIS5-S6 pore loop [Claes et al., 2001], were novel mutations.

The two nonsense mutations (c.7C>T and c.2796G>A) are located in the N-terminal part of SCN1A and within the DIIS5-S6 pore loop. These mutations, Q3X and W932X, result in a premature stop codon and predict a truncated protein of 2 and 931 amino acids, respectively. Five missense mutations occurred within an S5-S6 pore loop (c.1178G>A, c.2817C>G, c.2875T>C, c.4300T>C, c.5246G>A) and one in the DIVS4-S5 loop (c.4982T>C) (Fig. 1). The latter mutation (c.4982T>C; F1661S) corresponds to the previously reported mutation in the

human muscle channel SCN4A (F1473S), where it causes paramyotonia congenita. The mutant channel exhibits slowing of fast inactivation and acceleration of recovery from inactivation compared to wild-type channels [Mitrovic et al., 1996]. These data suggest the amino acid F1661 in SCN1A may have a similarly important role in the functioning of the neuronal Na⁺ channel SCN1A, and the F1661S mutation also potentially causes hyperexcitability. If this could be confirmed, SMEI in the patient with the F1661S mutation would be caused by a gain of function. So far, the missense mutations resulting in SMEI have not been studied in the in vitro expression systems. Lossin et al. [2002] introduced three GEFS+ mutations in human SCN1A (Na_v1.1) and examined the kinetic properties of the mutant channels in a mammalian expression system. They observed that all mutations disrupted channel inactivation, leading to hyperexcitability and suggesting a gain of function. The SMEI missense mutations should be studied in the human SCN1A, coexpressed with the human β-subunits, in a mammalian cell system as in the study of Lossin et al. [2002]. Only these functional studies will solve the apparent conflict between our previous hypothesis that SMEI is caused by loss of function and our current observation that missense mutations can also result in SMEI by a probable gain of function mechanism.

All other five SCN1A missense mutations in SMEI patients were located in regions of the channel that constitute the pore; the S5 and S6 segments are thought to be located near the pore, and the loop between S5 and S6 delineates the pore. In contrast, in GEFS+, SCN1A mutations have also been described in the loop between S2 and S3 [Wallace et al., 2001a], the loop between D2 and D3 [Escayg et al., 2001], and S2 [Abou-Khalil et al., 2001]. The region S4-S6 contains the voltage sensor (S4) and the pore region (S5-S6), which are functionally very important regions of the SCN1A channel. Therefore, it is not unlikely that specific missense mutations in the S4-S6

TABLE 3. SCN1A Sequence Variants

Position in SCN1A	DNA change	Major allele frequency
Intron 2	IVS2+66T>C	0.81 (T)
Exon 3	c.429G>A	0.97 (G)
Intron 4	IVS5_106T>G	0.63 (T)
Intron 4	IVS5_91G>A	0.56 (G)
Intron 7	IVS8_66T>C	0.63 (T)
Intron 8	IVS8+75C>T	0.84 (C)
Intron 8	IVS8+112C>T	0.56 (C)
Intron 8	IVS9_9_10delTT	0.91 (TT)
Exon 9	c.1212G>A	0.63 (G)
Intron 9	IV9+52G>A	0.56 (G)
Intron 9	IVS10_61delC	0.87 (C)
Intron 10	IVS11_47G>T	0.63 (G)
Exon 13	c.2292C>T	0.53 (C)
Intron 13	IVS14_72G>A	0.63 (G)
Intron 15	IVS16_41T>C	0.71 (T)
Intron 17	IVS18_90T>C	0.97 (T)
Intron 18	IVS18+33T>G	0.97 (T)
Intron 18	IVS18+55C>G	0.97 (C)

Numbering of sequence variants started from the initiating ATG codon as described by Escayg et al. [2000].

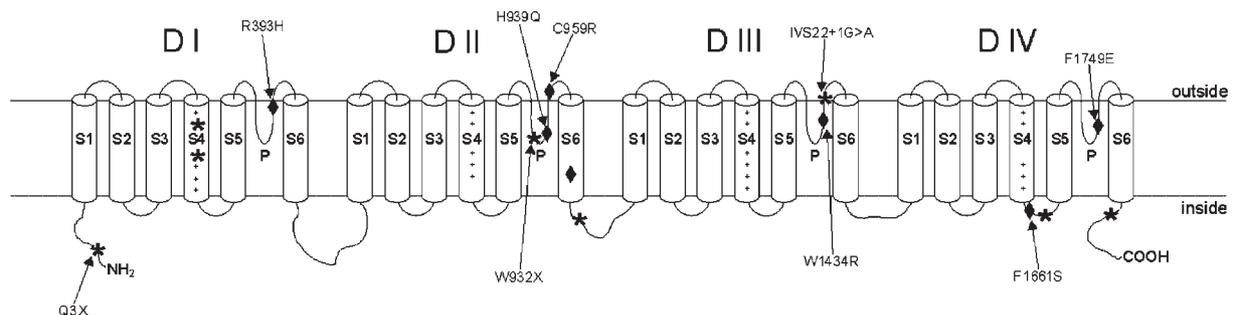


FIGURE 1. Presentation of SCN1A (Na_v1.1). The α-subunit of the voltage-gated neuronal sodium channel consists of four domains (DI-DIV), each comprising six transmembrane segments (S1-S6). The voltage sensor S4 is indicated with positive charges. P indicates the pore loop. SMEI mutations described in this article and our previous article [Claes et al., 2001] are indicated with a black diamond (◆, missense mutations) or an asterisk (*, nonsense and splice site mutations). An arrow indicates the position of the mutations identified in the present study.

region lead to serious alterations in the kinetic properties of the SCN1A channel and thus lead to the more severe phenotype observed in SMEI patients.

Recently, three Japanese groups reported on the mutation analysis results of SCN1A in their SMEI patients [Sugawara et al., 2002; Ohmori et al., 2002; Fukuma et al., 2002]. Sugawara et al. [2002] observed 10 SCN1A mutations in 14 SMEI patients (71%). All these mutations were truncating mutations; the de novo origin of the mutation was confirmed in four patients. Ohmori et al. [2002] observed 24 SCN1A mutations in 29 SMEI patients (83%). Interestingly, they identified a large number of missense mutations (10/24) in addition to 13 truncating mutations and one 3-bp deletion. Finally, Fukuma et al. [2002] identified 44 SCN1A mutations in 54 SMEI patients (82%). They reported 23 missense mutations and 11 truncating mutations. Apparently, several SMEI mutations were observed more than once, though information on their relative frequencies was not reported. A comparison of our two studies and the three Japanese studies raises several questions. First, why are there differences in the mutation rate of SCN1A in the different studies? In our studies, the mutation rate was 100% while that in the Japanese studies it ranged from 71% to 83%. Second, why were truncating mutations detected almost exclusively in some studies [Claes et al., 2001; Sugawara et al., 2002], while in other studies missense mutations occurred frequently in addition to truncating mutations [present study; Ohmori et al., 2002], or even outnumbered the truncation mutations [Fukuma et al., 2002]? Third, is there a difference in the clinical phenotype of patients with missense mutations or truncating mutations? The exact reason for the differences in mutation rate cannot be determined unambiguously. A major factor probably lies in the inclusion criteria, which might differ between the studies. In our studies, one experienced clinician selected all patients based on ILAE criteria. In addition, most patients were clinically followed for many years and displayed the complete SMEI phenotype including ataxia and mental retardation. It can be expected that inclusion in the screening of very young patients suspected of SMEI can result in a lower mutation rate because some of these patients will eventually develop a different epilepsy phenotype or additional symptoms, leading to a different diagnosis. However, in the study of Sugawara et al. [2002], several older patients with a fully developed SMEI phenotype did not have a SCN1A mutation in the coding region, suggesting other mutation mechanisms, such as large-scale deletions of the gene or mutations in the regulatory region. However, it is questionable whether these latter mutation mechanisms can explain the frequency of SMEI patients without a SCN1A mutation. Another obvious

explanation is the existence of genetic heterogeneity in isolated SMEI cases. The lower mutation rate in the latter situation, however, better reflects the mutation yield in a routine diagnostic laboratory, a situation that can differ substantially from ascertaining patients for a research project. Whether these or additional factors explain the different mutation rates observed in the different studies cannot be decided, due to lack of sufficient clinical information on the patients reported in some of the Japanese studies. The large number of missense mutations in our present study compared to our first one surprised us because we had used the same ILAE selection criteria for inclusion of SMEI patients. Therefore, the difference between the two studies is most likely coincidental. This is further supported by the observation that the clinical phenotype was not different for SMEI patients with truncating or missense mutations. In addition, Ohmori et al. [2002] did not find a correlation between clinical severity and type of SCN1A mutation.

In total, we identified 16 SMEI patients with a de novo mutation in SCN1A. Combined data from this study, our previous study [Claes et al., 2001], Sugawara et al. [2002], Ohmori et al. [2002], and Fukuma et al. [2002] suggest that mutations producing a truncated protein predominantly result in SMEI. In fact, nonsense or frameshift mutations associated with GEFS+ have not been reported. The effect of missense mutations is less predictable because they produce a variety of phenotypes, ranging from severe SMEI to mild GEFS+. This provides supportive evidence for the hypothesis of Singh et al. [2001], namely that SMEI is the most severe phenotype of the GEFS+ spectrum. Genotype–phenotype correlations indicated that the location within SCN1A of a missense mutation influenced the phenotypic outcome. Thus far, we identified missense mutations only in S4-S6 of SCN1A in SMEI patients, while missense mutations at different codon positions in S4-S6 were also reported in GEFS+, indicating that within S4-S6 certain amino acid substitutions are less critical than others for channel functioning. Important to mention are the findings of Ohmori et al. [2002]. In SMEI patients, they identified SCN1A missense mutations located in DIIIS2 and in the C-terminal region. The patient with the C-terminal region mutation had a less severe phenotype, also suggesting that the location of the mutation may influence the phenotype. Whether our findings that SMEI missense mutations occur mainly in the S4-S6 region of SCN1A are a coincidence or are truly biologically relevant can only be determined by further phenotype–genotype correlation studies.

In summary, in our study we identified nine de novo mutations in SCN1A in nine additional isolated SMEI patients. Along with our previous findings of de novo mutations in seven other SMEI patients [Claes et al., 2001], our data strongly support the hypothesis that

SMEI in isolated patients results from de novo mutations in *SCN1A*.

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