ORIGINAL CONTRIBUTION

Cryptogenic Epileptic Syndromes Related to *SCN1A*

Twelve Novel Mutations Identified

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Background: Sodium channel alpha 1 subunit gene, *SCN1A*, is the gene encoding the neuronal voltage-gated sodium channel α 1 subunit ($Na_v1.1$) and is mutated in different forms of epilepsy. Mutations in this gene were observed in more than 70% of patients with severe myoclonic epilepsy of infancy (SMEI) and were also found in different types of infantile epileptic encephalopathy.

Objective: To search for disease-causing mutations in *SCN1A* in patients with cryptogenic epileptic syndromes (ie, syndromes with an unknown cause).

Design: Clinical characterization and molecular genetic analysis of a cohort of patients.

Setting: University hospitals, rehabilitation centers, and molecular biology laboratories.

Patients: Sixty unrelated patients with cryptogenic epileptic syndromes.

Main Outcome Measures: Samples of DNA were analyzed for mutations and for large heterozygous deletions encompassing the *SCN1A* gene. A search for microdeletions in the *SCN1A* gene was also performed in the subset of patients with SMEI/SMEI-borderland who had negative results at the point mutation screening.

Results: No large deletions at the *SCN1A* locus were found in any of the patients analyzed. In contrast, 13 different point mutations were identified in 12 patients: 10 with SMEI, 1 with generalized epilepsy with febrile seizures plus, and 1 with cryptogenic focal epilepsy. An additional search for *SCN1A* intragenic microdeletions in the remaining patients with SMEI/SMEI-borderland and no point mutations was also negative.

Conclusions: These results confirm the role of the *SCN1A* gene in different types of epilepsy, including cryptogenic epileptic syndromes. However, large deletions encompassing *SCN1A* were not common disease-causing rearrangements in this group of epilepsies.

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SODIUM CHANNEL ALPHA 1 SUBunit gene (*SCN1A*) (MIM 182389, GenBank AB093548) is the gene most commonly mutated in different forms of epilepsy and it encodes the sodium channel α 1 subunit ($Na_v1.1$).[1] Mutations in this gene are observed in more than 70% of patients with severe myoclonic epilepsy of infancy (SMEI),[1-5] a syndrome with seizure onset in the first year of life and typically beginning with prolonged febrile hemiclonic or generalized tonic-clonic seizures.[2] Subsequently, other types of seizures occur, such as myoclonic, partial, and absence seizures, which are refractory to antiepileptic drug treatment. Psychomotor development is normal until seizure onset and then progressively slows down, leading to moderate to severe mental retardation.[5] More than 170 mutations of *SCN1A* are associated with SMEI[1,4] and SMEI borderland (SMEB).[4,6,7] Approximately 95% of *SCN1A* mutations arise de novo, and the remainder show familial segregation in generalized epilepsy with febrile seizures plus (GEFS+).[4,8] Mosaicism for *SCN1A* mutations has also been reported in unaffected parents of patients with SMEI or SMEB.[9-12] Besides SMEI, SMEB, and GEFS+, the phenotypic spectrum of *SCN1A* also includes intractable childhood epilepsy with generalized tonic-clonic seizures.[13] West syndrome is also associated with *SCN1A,* although only 1 mutation in 1 case has been reported so far.[14] Focal epilepsy, such as temporal lobe epilepsy, presenting occasionally within the GEFS+ spectrum, was associated with an *SCN1A* mutation as well.[15,16] A novel *SCN1A* mutation was also detected in a family with members affected by simple febrile seizures, with some of them later developing temporal lobe epilepsy.[17]

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It was recently found that intragenic and whole *SCN1A* gene deletions also occur in a variable percentage (8%-27%) of patients with SMEI without point mutations in *SCN1A*.[18-21] The overlapping yet heterogeneous clinical features of the epilepsy syndromes associated with *SCN1A* mutations recently led a group of researchers to search for mutations in this gene in a large sample of unselected patients with epileptic encephalopathies (including SMEI) with onset primarily during the first year of life.[4] In that study, they identified mutations in patients with cryptogenic generalized epilepsy (CGE) and cryptogenic focal epilepsy (CFE) and in a subgroup classified as having severe infantile multifocal epilepsy.

Based on these results, we decided to screen the *SCN1A* gene for mutations and whole gene deletions in 60 patients with cryptogenic epileptic syndromes with onset in the first 2 years of life to evaluate the prevalence of *SCN1A* abnormalities in this heterogeneous group of patients. All the patients with SMEI and SMEB who had negative results at the point mutation screening were also analyzed for the presence of intragenic heterozygous deletions in the *SCN1A* gene. The results of these screenings are presented and discussed.

METHODS

PATIENTS

Sixty patients (59 from Italy and 1 from Spain), selected based on the criteria detailed herein, were referred for molecular analysis to the Laboratory of Molecular Biology at the E. Medea Scientific Institute, Lecco, Italy. These patients were first seen with an epileptic syndrome of unknown cause, which started in the first 2 years of life; this diagnosis was based on normal magnetic resonance imaging findings and negative results of metabolic and cytogenetic investigations.

Each patient has been followed up for a variable period (2-28 years) since the onset of the epileptic seizures. Clinical data were obtained from all the patients, and particular attention was given to family history of seizure disorders; seizure onset, type, frequency, and precipitating factors; drug response; and evolution of electroclinical data. Several polygraphic-polysomnographic electroencephalograms were recorded. The patients enrolled in this study could, thus, be classified into 6 different subgroups according to the International League Against Epilepsy classification of epileptic syndromes[22] and the International League Against Epilepsy proposed diagnostic scheme for epilepsies[23]: SMEI (n=14), SMEB (n=6), GEFS+ (n=12), West syndrome (n=7), CFE and multifocal epilepsies (n=8), and CGE (n=13). The term *SMEB* was used for cases of SMEI without several key features of SMEI.[24] All the patients with West syndrome enrolled in this study were negative for mutations in the genes known for this disease (aristaless-related homeobox [MIM 300382, GenBank NM_139058] and cyclin-dependent kinase-like 5 [MIM 300203, GenBank NM_003159]). The CGE subgroup comprises 4 cases of Lennox-Gastaut syndrome. The ethics committee of the E. Medea Scientific Institute approved the study. Informed consent was obtained from the parents of children and from adults of normal intellect.

MOLECULAR ANALYSIS

Molecular analysis was performed on genomic DNA extracted from blood using standard procedures. All 26 exons of *SCN1A* were amplified by polymerase chain reaction (PCR) using flanking intronic primers and standard PCR conditions. The PCR fragments were sequenced using a kit (BigDye Terminator Sequencing Kit; Applied Biosystems, Foster City, California) and were run on a genetic analyzer (ABI 3130 XL; Applied Biosystems). The *SCN1A* mutation nomenclature is based on RefSeq AB093548.1 (considering the A of the ATG as nt 1) according to the recommendations of the Human Genome Variation Society. The appropriate PCR fragment from parents' DNA (where available) was sequenced in all cases in which an *SCN1A* mutation was detected to distinguish between de novo and familial variants. All the reported nucleotide changes were checked in a panel of 250 control subjects. Quantitative PCR was performed using probes designed on intron 3 and exons 8 and 26 of the *SCN1A* gene. The reactions were run on a detection system (ABI 7900HT Sequence Detection System; Applied Biosystems). Multiplex ligation-dependent probe amplification analysis was performed using a kit (SALSA MLPA P137 Kit; MRC-Holland, Amsterdam, the Netherlands). Possible changes in exonic splicing enhancers were assessed using the exonic splicing enhancer finder algorithm (accessible at http://rulai.cshl.edu/tools/ESE).

RESULTS

Of the 60 patients analyzed, 12 were found to carry mutations in *SCN1A*: 10 with SMEI, 1 with GEFS+, and 1 with CFE. No mutations were found in the remaining epilepsy subgroups. The clinical features of patients with mutations are summarized in **Table 1** and described in the following subsections, grouped by the type of epilepsy syndrome.

SEVERE MYOCLONIC EPILEPSY OF INFANCY

All patients with mutations were born after an uneventful pregnancy and delivery. Epilepsy started at 3 to 8 months of life in all patients with febrile (n=8) or afebrile (n=2) generalized (n=5) or hemiclonic (n=5) seizures changing sides. Prolonged seizures or status epilepticus was reported in all the patients except 3. Seven patients started experiencing segmental or massive myoclonus jerks after 12 months of age; 4 developed partial seizures and 5 experienced atypical absence seizures; 1 patient had tonic seizures. At the neurologic examination, 4 patients developed ataxia. Five patients had severe mental retardation, while 4 showed only mild cognitive deficits. Each patient was treated with more than 3 antiepileptic drugs without achieving complete seizure control.

CRYPTOGENIC FOCAL EPILEPSY

One patient had a family history of unspecified epilepsy in a maternal uncle, although both parents were healthy. The delivery was complicated, and the patient has had mild psychomotor retardation since the first year of life, with lateralized (right) neurologic signs and a left ptosis observed at age 10 months. Epilepsy started at age 3 months with generalized tonic-clonic seizures. Hemiclonic seizures involving only the left side subsequently occurred, sometimes followed by febrile and afebrile convulsive status epilepticus. This patient is now severely

Table 1. Clinical and Electroclinical Features of Patients With *SCN1A* Mutations

Feature	Patient No. 1	2	3	4	5	6	7	8	9	10	11	12
Age at diagnosis, y	15	3	28	29	15	12	9	5	2	2	17	7
Epileptic syndrome	SMEI	SMEI	SMEI	CFE	SMEI	SMEI	SMEI	SMEI	GEFS+	SMEI	SMEI	SMEI
Family history	+	–	–	+	–	+	–	–	NA	–	–	–
Seizures												
Age at onset, mo	5	5	4	3	5	5	<12	8	<12	6	<6	4
Type	H	GTC	GTC	GTC	GTC	H	H	H	GTC	GTC	GTC	H
Febrile	+	+	+	+	+	–	+	–	+	+	+	+
Other type	GTC	ABS	ABS, tonic, and partial	H[a] and partial	–	ABS and H	Partial	Partial	–	–	ABS	Partial and ABS
Frequency	Daily	Monthly	Monthly	Weekly	NA	Weekly	Weekly	Weekly	Yearly	Monthly	Monthly	Weekly
Status epilepticus	+	+	+	+	–	–	NA	NA	–	+	NA	+
Myoclonus	+	–	+	+	–	+	+	+	–	–	+	+
Treatment	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Resp	Ref	Ref	Ref
Neurologic examination	NAD	NAD	Ataxia	Ataxia	Bab	NAD	Ataxia	Ataxia	NAD	NAD	NAD	Ataxia
MR	S	MI	S	S	S	M	S	M	None	M	S	M
MRI	N	N	N	N	N	N	N	N	N	N	N	N
EEG background in childhood	N	NA	SI	SI	SI	SI	N	SI	N	N	NA	SI
Photoparoxysmal response	+	+	–	–	–	+	–	–	–	–	NA	+

Abbreviations: ABS, absence seizures; Bab, Babinski; CFE, cryptogenic focal epilepsy; EEG, electroencephalographic; GEFS+, generalized epilepsy with febrile seizures plus; GTC, generalized tonic-clonic; H, hemiclonic; M, moderate; MI, mild; MR, mental retardation; MRI, magnetic resonance imaging; N, normal; NA, not available; NAD, nonabnormal data; ref, refractory; resp, responsive; S, severe; SI, slowly; SMEI, severe myoclonic epilepsy of infancy; +, present; –, absent.

[a] Always on the same side (see the "Results" section).

mentally retarded. Electroencephalograms revealed slowed background activity and bursts of epileptiform abnormalities bilaterally over the frontal areas.

GENERALIZED EPILEPSY WITH FEBRILE SEIZURES PLUS

The family history for this patient was unavailable. The patient's parents agreed to donate blood for genetic analysis but refused to be clinically evaluated. The patient was born after an uneventful pregnancy and delivery. Psychomotor development was normal. Epilepsy started in the first year of life with febrile generalized tonic-clonic seizures. Generalized afebrile tonic-clonic seizures appeared at age 3 years. He has never experienced prolonged seizures or status epilepticus. The patient is now 6 years old, and the seizures are well controlled with sodium valproate therapy. His intelligence and neurologic examination results are normal.

MOLECULAR ANALYSIS

Mutation screening of *SCN1A* performed by direct sequencing of all exons led to the identification of 13 mutations in 12 patients (**Table 2**). Two truncating mutations in exon 1 (p.Y65X) and exon 22 (p.W1434X) and 8 missense mutations distributed in exon 2 (p.R118S), exon 8 (p.D366E and p.R377Q), exon 18 (p.L1207P), exon 21 (p.V1335M and p.V1358S), exon 23 (p.Y1462C), and exon 26 (p.R1928G) were found. Mutations p.L1207P and p.R1928G were present in the same patient (Table 2). Frameshift mutations due to a single base deletion in exon 19 (c.3774delA) or a 4–base pair deletion in exon 26 (c.5536_5539delAAAC) were also identified in 2 patients. The 2 deletions cause premature stop codon 63 and 10 base pairs downstream (p.L1296fs and p.K1846fs), respectively. A single splice mutation affecting the donor splice site of exon 8 (c.1170 + 1 G→A) was identified in 1 patient. No cell line from the patient was available to check the mutation effect on gene transcription; however, the base change most likely leads to either exon skipping or use of a cryptic splice donor site. All the mutations identified are novel except for c.5536_5539delAAAC, which has already been reported.[1,3,4,14,25] None of the novel mutations were present in a panel of 250 controls. The mutations are scattered throughout the $Na_v1.1$ subunit protein, with the S5-S6 segments (the pore-forming regions) of 2 domains, DI and DIII, being the more frequent targets (Table 2). Most of the mutations found, which could be tested for segregation within the families (8 of 9), turned out to be de novo. In addition to the point mutation screening, we wanted to exclude the presence of large heterozygous deletions encompassing the entire *SCN1A* gene in all point mutation–negative patients. Quantitative PCR was, thus, performed by using probes specific for intron 3 and exons 8 and 26 of the *SCN1A* gene. No large heterozygous *SCN1A* deletions could be found in any of the patients tested. The *SCN1A* intragenic microdeletions were also excluded by using the multiplex ligation-dependent probe amplification technique only in patients with SMEI/SMEB who had negative results at point mutation screening.

Table 2. *SCN1A* Mutations

Patient No./ Phenotype	Nucleotide Change	Amino Acid Change	Exon/ Intron	ESE Change	*SCN1A* Domain	Inheritance
2/SMEI	c.195T>A	p.Y65X	1	NA	NH2 ter	De novo
1/SMEI	c.354G>C	p.R118S	2	Yes	NH2 ter	De novo
10/SMEI	c.1098T>A	p.D366E	8	Yes	DI-S5-S6	De novo
9/GEFS+	c.1130G>A	p.R377Q	8	Yes	DI-S5-S6	Maternal
11/SMEI	c.1170+1 G>A	NA	IVS8	NA	DI-S5-S6	De novo
3/SMEI	c.3774delA	p.L1269fs	19	NA	DIII-S2	De novo
5/SMEI	c.4003G>A	p.V1335M	21	Yes	DIII-S4-S5	ND
8/SMEI	c.4073G>C	p.W1358S	21	No	DIII-S5	De novo
7/SMEI	c.4301G>A	p.W1434X	22	NA	DIII-S4-S5	De novo
6/SMEI	c.4385A>G	p.Y1462C	23	No	DIII-S6	ND
4/CFE	c.5536_5539delAAAC[1,3,4,14,25]	p.K1846fs	26	NA	COOH ter	De novo
12/SMEI	c.5782C>G	p.R1928G	26	Yes	COOH ter	ND
	c.3620T>C	p.L1207P	18	No	DII-DIII linker	

Abbreviations: CFE, cryptogenic focal epilepsy; COOH ter, COOH terminus; ESE, exonic splicing enhancer; GEFS+, generalized epilepsy with febrile seizures plus; NA, not available; ND, not determined; NH2 ter, NH2 terminus; SMEI, severe myoclonic epilepsy of infancy.

COMMENT

Sixty patients with cryptogenic epileptic syndromes characterized by seizure onset in the first 2 years of life were tested for point mutations and whole gene deletions in *SCN1A*. No large deletions including the *SCN1A* gene were found in any patients, indicating that these rearrangements are not frequently associated with cryptogenic epileptic syndromes. However, 13 *SCN1A* point mutations were detected in 12 patients. Most of these patients have SMEI (10 of 12), with the remaining having GEFS+ and CFE.

SEVERE MYOCLONIC EPILEPSY OF INFANCY

The large percentage of SMEI in patients with *SCN1A* mutations was expected based on the reported data.[1,4] In the present study, 10 (71%) of 14 patients with SMEI analyzed showed *SCN1A* mutations, and this is in line with the results obtained in the most recently reported large patient sample screenings.[1,4] One patient with SMEI carried 2 heterozygous mutations in exons 18 and 26 of *SCN1A*. Both changes affect evolutionary conserved residues and fall within gene regions representing frequent targets of mutations. However, we cannot exclude that at least 1 of the changes represents a rare variant. These data, together with the results of the deletion screening, indicate that *SCN1A* mutation detection in patients with SMEI is still lower than 100%. This reinforces the idea that the molecular genetic basis of SMEI is not fully known yet, as was previously hypothesized.[1]

GENERALIZED EPILEPSY WITH FEBRILE SEIZURES PLUS

We observed an *SCN1A* mutation in 1 GEFS+ patient (patient 9) of the 12 analyzed (8%). This percentage is consistent with the reported frequency of *SCN1A* mutations in families with GEFS+ (5%-10%).[1] Other genes known to be involved in this type of epilepsy, such as *SCN1B*, *SCN2A*, and *GABRG2,* still need to be screened in this sample.

CRYPTOGENIC FOCAL EPILEPSY

Unlike in the results of a recent study,[4] only 1 of 8 patients with CFE in our study had an *SCN1A* mutation (12.5% vs 22%). The only mutation found was the c.5536_5539delAAAC deletion, which has been reported so far in 4 other patients with a different clinical diagnosis. The patient described herein is classified as having CFE owing to the presence of focal electroencephalographic abnormalities, hemiclonic seizures occurring always on the same side, and lateralized neurologic deficits. Three patients already described with this mutation had SMEI,[3,14,25] and a fourth was defined as having SMEB without generalized spike waves.[4] The identification by different researchers of the same mutation in apparently different clinical phenotypes might be due to either a blurred clinical distinction between these epileptic syndromes or other genetic or environmental factors playing a role in the expression of the epilepsy phenotype. This idea would be consistent with the marked variability widely reported in patients with SMEI and related parents carrying the same mutation and with the hypothesis of a polygenic origin of SMEI, as previously suggested.[1] Functional data on the mutant protein will contribute to addressing this issue.

The negative results obtained in the other subgroups of patients, such as the West and Lennox-Gastaut syndromes subgroups and the CGE subgroup, are only partly unexpected. Indeed, the previous identification of only 1 *SCN1A* mutant case in the West and Lennox-Gastaut syndromes,[4,14] together with the present negative results, indicates that *SCN1A* is a rare cause of disease in these syndromes. These findings also suggest that an *SCN1A* mutation search may not be necessary in routine diagnostic practice for these forms of epilepsy, but rather that it should be considered for research purposes.

Overall, the negative results at the *SCN1A* mutation screening in the CGE subgroup, compared with the remarkable percentage (24%) of patients with *SCN1A* mutations in the CGE subgroup analyzed by Harkin and colleagues,[4] might be explained by the likely clinical heterogeneity between the 2 CGE subgroups tested. The

same reason may explain the different percentages of *SCN1A* mutants in the CFE subgroups as well. Such heterogeneity may originate from the different types of epilepsy examined in the 2 studies: the severe epileptic encephalopathies with onset in the first year of life in the first study[4] vs the cryptogenic epileptic syndromes with onset in the first 2 years of life tested herein. Based on this, the present data seem to indicate that the earlier and more severe the clinical and electroencephalographic presentations are, the higher the probability of finding mutations in *SCN1A* is. However, additional experimental evidence is required to confirm this hypothesis.

SCN1A MUTATIONS

Twelve of the 13 *SCN1A* mutations identified in this study are novel, thus supporting the concept of mutational heterogeneity that is typical of *SCN1A*. Most of the mutations found (8 of 9 tested) were de novo, consistent with the data in the literature.[4]

Seven of the 13 mutations found, including missense, nonsense, and deletions, fall within the S5-S6 segments of the DI and DIII domains of the $Na_v1.1$ subunit. Whereas the pathogenic effect of a truncating mutation is clear, the postulated pathogenicity of missense mutations falling within these segments needs functional demonstration. These segments represent the pore-forming region of the channel subunit; therefore, any change affecting the properties of the constituting residues, such as the charge or the steric hindrance (as in mutations p.V1335M and p.Y1462C) or both (as in mutations p.R377Q and p.W1358S), is supposed to variably affect channel activity. In addition, all the identified missense mutations change an evolutionary conserved amino acid residue, and all but 3 (p.W1358S, p.Y1462C, and p.L1207P) determine a putative exonic splicing enhancer sequence change. These sites are well-known to play a role in constitutive and alternative splicing events.[26]

In conclusion, this study represents the second systematic study of *SCN1A* mutations in cryptogenic epilepsies and confirms the findings of the first study[4] conducted on a larger sample of patients, although with different results for some types of epilepsy. The results of these 2 studies are supported and completed by the previous findings of *SCN1A* mutations in a condition previously considered to be symptomatic, such as the alleged vaccine encephalopathies (mutations found in 11 of 14 patients)[27] or in adult cases with refractory epilepsy and normal magnetic resonance imaging findings with onset in infancy (10 of 14 patients).[5]

These data altogether extend the spectrum of the clinical phenotypes associated with *SCN1A* mutations to include SMEI and other epileptic encephalopathies and different types of cryptogenic epilepsies with clear diagnostic implications. All these studies are actually single pieces of an apparently big puzzle representing the wide and heterogeneous spectrum of pathologic manifestations associated with $Na_v1.1$ subunit dysfunctions.

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