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Brief Communication

Lack of SCN1A Mutations in Familial Febrile Seizures

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Summary: *Purpose:* Mutations in the voltage-gated sodium channel subunit gene SCN1A have been associated with febrile seizures (FSs) in autosomal dominant generalized epilepsy with febrile seizures plus (GEFS$^+$) families and severe myoclonic epilepsy of infancy. The present study assessed the role of SCN1A in familial typical FSs.

Methods: FS families were selected throughout a collaborative study of the Italian League Against Epilepsy. For each index case, the entire coding region of SCN1A was screened by denaturant high-performance liquid chromatography. DNA fragments showing variant chromatograms were subsequently sequenced.

Results: Thirty-two FS families accounting for 91 affected individuals were ascertained. Mutational analysis detected a single coding variant (A3169G) on exon 16. The extended analysis of all family members and 78 normal controls demonstrated that A3169G did not contribute to the FS phenotype.

Conclusions: Our study demonstrated that SCN1A is not frequently involved in common FSs and suggested the involvement of specific FS genes. **Key Words:** Febrile convulsions—Idiopathic epilepsy—Ion channels—Genetics—Mutations.

Recent progress in epilepsy research has shown that different mendelian idiopathic epilepsies are associated with mutations in genes encoding for neuronal ion-channel subunits.

Among these, mutation of voltage-gated sodium channel subunits genes SCN1A and SCN1B have been found in some families showing generalized epilepsy with febrile seizures plus (GEFS$^+$) and in sporadic cases of severe myoclonic epilepsy of infancy (1–3).

The GEFS$^+$ syndrome is characterized by a heterogeneous phenotype segregating as an autosomal dominant trait with incomplete penetrance. In GEFS$^+$, missense mutations in SCN1A-SCN1B underlie different forms of afebrile seizures such as absence, myoclonic, myoclonic–astatic, and generalized tonic–clonic seizures and FSs of either the typical or "plus" type.

Although most seizures occurring in GEFS$^+$ families are nonspecific symptoms found in many epileptic syndromes, the presence of individuals showing FSs per-

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sisting beyond age 6 years (defined as "plus") and frequently associated with afebrile seizures defines a peculiar phenotype (from which the name of the syndrome was derived).

However, genotype–phenotype analyses of GEFS$^+$ families show that ~50% of individuals carrying mutations in SCN1A and SCN1B manifest typical FSs (1,2,4–7).

A further confirmation of the primary role of voltage-gated sodium channel genes in the pathogenesis of FSs was provided by a recent study showing de novo SCN1A mutations in sporadic cases affected by severe myoclonic epilepsy of infancy (SMEI), a rare form of epilepsy characterized by FSs, myoclonic seizures, and different types of generalized seizures and psychomotor delay (3). In SMEI, the complex epileptic phenotype is due to frameshift, nonsense, and splice-site mutations leading to protein truncation and subsequent haploinsufficiency.

So far febrile seizures appear the most common clinical feature resulting from mutations in SCN1A and SCN1B. Consistent work has been carried out to determine the role of SCN1B in typical FSs, and no mutations were found in 25 FSs families (2). In contrast, the important issue of assessing the contribution of SCN1A to typical FSs affecting ≤5% of children has not yet been addressed.

METHODS

We screened 32 FSs multiplex families of Italian descent for SCN1A mutations. Diagnosis of FS followed the criteria established in the 1989 International Classification of Epileptic Syndromes. Anamnestic data and electroencephalographic (EEG) recordings were collected for all family members including healthy parents and first-degree relatives of the affected patients. The EEG was normal for all patients or showed mild aspecific abnormalities. Criteria for inclusion of families included the presence of at least an affected sib pair with one or no affected parents.

A family with members with (a) afebrile seizures, (b) febrile seizures at older than 6 years (febrile seizures plus), (c) epileptiform EEG traits and showing bilineal inheritance of FSs was not included in the study.

Peripheral blood was obtained from consenting individuals and used for the establishment of lymphoblastoid cell lines by Epstein–Barr virus (EBV) transformation. Genomic DNA was obtained from patients' lymphoblasts by using standard protocols.

Genomic organization of SCN1A was deduced by human SCN1A sequence reported by Escayg et al. (1) and genomic sequence AC010127.

Mutational analysis was performed by denaturant high

TABLE 1. *Primers used to amplify SCN1A exons*

Exon	Primer (5′ → 3′) Forward	Primer (5′ → 3′) Reverse	Size (bp)	T[a] ann.
1	ATG TGT TGG TGC TAC AAC AGT CC	AAA ACA GAC TTT AAA TCC TCT AGC	493	62°C
2	ATT TGA TAT TTA GCT ATA AAG TGC	AGT AGA TAA CAG AGT TTA AGT GG	256	56°C
3	TTG CTG CGT AAT TTT GTC TAG G	AGT TTG GGC TTT TCA ATG TTA GC	302	60°C
4	TAT TCT ACA GGT AAA GCA AAC C	GAG TGA TAA GAA ATT GGT ATG C	296	60°C
5	AAA CAC CTA GTC TTA TGA TTC C	ATA GGC TCT TTG TAC CTA CAG C	271	60°C
6	GGA TAT CCA GCC CCT CAA GT	TGC TTC TCC ACT AGC GTT GC	488	60°C
7	TAA CAA TGC AAA TGT TCA TCA TA	TAA TCT CAT ACT TTA TCA AAA ACC	263	58°C
8	GAG TAA AAA GGC AGC AGA ACG	CAA GTC TCG TTT CAA GTT CTG C	315	60°C
9	TTG AAA GTT GAA GCC ACC AC	TCC TCA TAC AAC CAC CTG CTC	374	60°C
10	AAG CCA TGC AAA TAC TTC AGC	TTC TAA TTC TCC CCC TCT CTC C	438	60°C
11	TCT GTT ATG AAT GCT GAA ATC TCC	CTG GTG CAG CAA TAG TGA CG	541	60°C
12	GTC ACC ATT TGG TTG TTT GC	TGC ACT ATT CCC AAC TCA CAA	299	60°C
13	TGA AAA TAA GTT TAG TGG ATA TG	CAG GAA GCA TGA AGG ATG GT	421	60°C
14	AGA ATC ATT GTG GGA AAA TAG C	ACA ATG CTA ATG GTT GTG TGG	350	60°C
15	ATG AGC CTG AGA CGG TTA GG	TGC CAT GCT GGT GTA TTT CC	537	60°C
16a	TCA TCA AGT TTT AGA ACT TAG AG	TTC AAC ACT GCT GCC AGT TC	449	60°C
16b	TGG ATA GGA TGC ACA AAG GA	GCT GAG GAT CAT CTG TAT GTG TG	459	60°C
17	TTG GCA GGC AAC TTA TTA CC	CTG ACC AAC AGC TAA ACA AGC	247	60°C
18	CAA GAC AAG GAC ATT GCT AAA GG	AAT AAG TCA TCA GTA TTA GAG TG	298	60°C
19	CTG CCC TCC TAT TCC AAT GA	CAA GCT ACC TTG AAC AGA GAC AAA	325	60°C
20	AAT GTC TGA ACA TTT ATC CTC TG	AAG AAT TTG CCA TTC CTT TTG C	349	60°C
21	TGG AAA GAC CAG AGA TTA CTA GGG	TAT GTC ATT ATT TTG TTA TTA TTC C	434	60°C
22	TGT CTT GGT CCA AAA TCT GTG	TGG TCG TTT ATG CTT TAT TCG	282	60°C
23	ACC AGT GAC ATT TCC AGC AC	TTT GGC AGA GAA AAC ACT CC	271	60°C
24	TGT ACA AAA GGA CAC AGT TTT AAC C	TTT TTT CTA CTG GAA ATG TTA GC	252	60°C
25	GCT AAT CGA CAT GAG AAA ACT CC	GAA TCT AAT CTT GAT TGT TTG AGC	397	60°C
26b	AAA AAT ACA TCA CCT TCA CAG G	CCA ATC CTT CCA AGG TCT CC	408	60°C
26b	AAG CAC GCT GAA TAA TGA CAG C	GCT CAG TTA AGG GAG ACT GTG G	528	60°C
26c	GTT CAC CAC AAC CAG GAA GG	AAC GCA TGA TTT CTT CAC TGG	532	60°C

[a] Annealing temperature used for polymerase chain reaction amplifications.

TAB E 2. *Summary description of febrile seizure families*

	No. families	Affected members					Affected/ family
		Total	Sib twins	Sib triplets	Sib quadruplets	Parents	
Nuclear	29	77	24	4	1	13	2.7
Three-generation	3	14	2	2	—	4	4.7
Total	32	91	26	6	1	17	2.8

performance liquid chromatography (DHPLC) and sequencing of polymerase chain reaction (PCR) products showing variant chromatograms. Twenty-nine PCR fragments from 26 exons of SCN1A were individually amplified from genomic DNA of probands by using intronic primers (Table 1). Genomic DNA of two unaffected individuals was sequenced to obtain polymorphism-free standard DNA. Patients' fragments were loaded on the Wave System (Transgenomic, Inc.) with and without the corresponding fragment obtained from standard DNA to detect both heterozygous and homozygous variations.

Fragments showing variant chromatograms were sequenced and analyzed on the 377 ABI prism Genetic Analyzer.

Case–control analysis was carried out with Fisher's Exact test. TDT analysis was performed with the S-TDT program 1.1.

RESULTS

Thirty-two multiplex families accounting for 91 affected individuals were selected for a multicentric study. The familial structure of the selected sample is described in Table 2.

Among 91 FS patients, 84 (92%) showed simple FSs, and seven (8%) had complex FSs. At the last follow-up examination 38% of patients were older than 15 years, 30% were between 10 and 14 years, and 32% were younger than 10 years. Sex ratio (F/M) was 0.72.

A total of 35 individuals (the index case of each of 29 nuclear families plus two cases for each three-generation family) was screened for mutations. Ten nucleotide variants were found: one coding polymorphism (A3199G) in exon 16, encoding amino acid substitution T1067A; two silent exonic polymorphisms, G1212A in exon 9 and C2292T in exon 13; and seven polymorphisms on introns 2, 6, 7, 8, 13 (two), and 23. All of them were previously reported and allele frequencies estimated in a European population (7).

As it was the only coding polymorphism identified in our family sample, we typed 78 normal controls for A3199G and performed a case–control study to determine whether amino acid substitution T1067A is involved in the etiology of FSs. The frequency of the A allele was 65.7% in the affected subgroup and 63.5% in normal controls. The difference was not statistically significant, as shown by the Fisher's Exact test ($p = 0.43$). We typed all family members and performed association analysis for A3199G by the sib transmission–disequilibrium test. No evidence of association was detected for any of the alleles ($p = 0.09$).

CONCLUSIONS

Voltage-gated sodium channel subunit gene SCN1A has been associated with a broad phenotypic spectrum including typical benign FSs, afebrile generalized seizures, and SMEI. However, genotype–phenotype correlations suggest that FSs are the most common clinical features associated with SCN1A mutations. To determine whether SCN1A is involved in common FSs, we screened 35 patients belonging to 32 FS multiplex pedigrees. We found a single coding variant on exon 16 of SCN1A (A3199G) that is not associated with FSs in our family sample, as it was demonstrated for idiopathic generalized epilepsy (7).

Together with a previous study, our data suggest that genetic factors other than SCN1A and SCN1B are involved in the etiology of common typical familial FSs. This finding is consistent with a recent study demonstrating that SCN1A does not contribute to the etiology of common forms of idiopathic generalized epilepsies (7). Thus, although sharing important clinical features, pure FSs and FSs associated with idiopathic generalized epilepsy and pure idiopathic generalized epilepsies may not share a common genetic etiology.

The very recent identification of mutations in the γaminobutyric acid (GABA) receptor γ_2 subunit gene (GABRG2) and SCN2A segregating in families showing a combined FS–idiopathic generalized epilepsy phenotype confirms the complex pathogenesis of FSs in human epilepsy (8–10). Further studies will be required to determine the role of these genes in typical FSs and in idiopathic generalized epilepsy.

Furthermore, loci for FSs have been mapped on chromosomes 5q, 8q, and 19p in autosomal dominant FSs (11–13), whereas digenic inheritance was suggested for a pedigree segregating FS and temporal lobe epilepsy and loci mapped to chromosome 8q and 18qter (14).

The genetic etiology of the most common forms of FSs is therefore unknown. From this perspective, future studies could be focused on the analysis of available candidate genes such as other neuronal voltage-gated sodium channel genes or GABA-receptor genes and on

confirmation analysis of FS loci that have been mapped in the genome in the last decade.

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