Am. J. Hum. Genet. 68:859–865, 2001

Neuronal Sodium-Channel α1-Subunit Mutations in Generalized Epilepsy with Febrile Seizures Plus

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Generalized epilepsy with febrile seizures plus (GEFS+) is a familial epilepsy syndrome characterized by the presence of febrile and afebrile seizures. The first gene, *GEFS1*, was mapped to chromosome 19q and was identified as the sodium-channel β1-subunit, *SCN1B*. A second locus on chromosome 2q, *GEFS2*, was recently identified as the sodium-channel α1-subunit, *SCN1A*. Single-stranded conformation analysis (SSCA) of *SCN1A* was performed in 53 unrelated index cases to estimate the frequency of mutations in patients with GEFS+. No mutations were found in 17 isolated cases of GEFS+. Three novel *SCN1A* mutations—D188V, V1353L, and I1656M—were found in 36 familial cases; of the remaining 33 families, 3 had mutations in *SCN1B*. On the basis of SSCA, the combined frequency of *SCN1A* and *SCN1B* mutations in familial cases of GEFS+ was found to be 17%.

Introduction

Generalized epilepsy with febrile seizures plus (GEFS+ [MIM 604236]) was first described by Scheffer and Berkovic (1997) and is now recognized as a common epilepsy syndrome (Baulac et al. 1999; Moulard et al. 1999; Peiffer et al. 1999; Singh et al. 1999; Scheffer et al. 2000). Although GEFS+ is familial, it was initially difficult to recognize it as a distinct syndrome, because of clinical heterogeneity within each family. The common phenotypes are typical febrile seizures (FS) and febrile seizures plus (FS+); FS+ differs from FS in that the attacks with fever continue beyond age 6 years and/or include afebrile tonic-clonic seizures. Less common phenotypes include FS+ associated with absences, myoclonic or atonic seizures, and even more-severe syndromes, such as myoclonic-astatic epilepsy. That such phenotypic diversity could be associated with the segregation of a mutation in a single gene was established by the identification of a mutation in the voltage-gated sodium-channel β1-subunit gene, *SCN1B* (Wallace et al. 1998). This mutation, C121W, changes a conserved cysteine residue, disrupting a putative disulfide bridge, which results in in vitro loss of function of the β1-subunit. Without a functional β1-subunit, the rate of inactivation of sodium-channel α-subunits decreases, which may cause increased sodium influx and thereby result in a more depolarized membrane potential and hyperexcitability. Modifier genes or the environment may interact with the *SCN1B* gene to account for clinical heterogeneity, but the rarity of *SCN1B* mutations (Wallace et al. 1998) strongly suggests that additional genes of large effect underlie GEFS+ in other families (Singh et al. 1999).

GEFS+ in four families has been mapped to chromosome 2q (Baulac et al. 1999; Moulard et al. 1999; Peiffer et al. 1999; Lopes-Cendes et al. 2000). Recently, mutations in the neuronal voltage-gated sodium-channel α1-subunit gene, *SCN1A*, were described in two of these families (Escayg et al. 2000). These mutations, T875M and R1648H, are located in highly conserved S4 transmembrane segments of the channel, which are known to have a role in channel gating. It was suggested that these mutations may reduce the rate of inactivation of *SCN1A* and therefore may have an effect similar to that of the β1-subunit mutation.

GEFS+ is clearly a common complex disorder, with a strong genetic basis, incomplete penetrance, and genetic and phenotypic heterogeneity. FS occur in 3% of

Received December 20, 2000; accepted for publication January 30, 2001; electronically published March 13, 2001.

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0002-9297/2001/6804-0008$02.00

the population, and thus this phenotype may occur sporadically in families with GEFS+, in addition to occurring as a result of the GEFS+ gene (Wallace et al. 1998). Also, although some families segregate an autosomal dominant gene of major effect, in many cases the clinical genetic evidence (such as bilineality) suggests that in some small families the disorder is multifactorial (Singh et al. 1999). Despite this evidence, large families continue to be ascertained, and, with critical phenotypic analysis, they provide opportunities to localize and, ultimately, identify the genes involved.

The present article describes the genomic organization of *SCN1A* and the frequency of mutations in 53 unrelated patients with GEFS+. Three novel *SCN1A* mutations were identified, giving GEFS+-associated *SCN1A* mutations a frequency of 5.6%. Several *SCN1A* polymorphisms are also identified, which may prove useful in future association studies.

Subjects and Methods

Subjects

A group of 53 unrelated probands with GEFS+ phenotypes were studied. These subjects were ascertained on the basis of twin and family studies and on the basis of routine clinical practice. Phenotypes in probands and family members were classified as described elsewhere (Scheffer and Berkovic 1997; Singh et al. 1999). Familial cases ($n = 36$) were those in which at least one first-degree relative of the proband had a phenotype within the GEFS+ spectrum. Informed consent was obtained from all subjects.

The Australian family in figure 1*A*, which has been described extensively elsewhere (Scheffer and Berkovic 1997; Lopes-Cendes et al. 2000), is the original pedigree leading to the initial delineation and description of the GEFS+ syndrome. The Israeli family in figure 1*B* is of Ashkenazi origin and spans six generations. Twelve family members had seizures. In the two oldest members—I-2 and III-3—seizures had occurred in childhood, but the data were insufficient to allow classification of the phenotype. Of the 10 other family members who had seizures, 3 had FS with onset at age 9–13 mo and that terminated by age 1–4 years, with 1–7 attacks each, and five had FS+ with onset at age 9–24 mo and that terminated at age 5–41 years, with 2–15 attacks each. Seizures during childhood were a mixture of FS and afebrile tonic-clonic seizures, whereas the rarely occurring seizures during teenage and adult years were all afebrile. Subject V-16 had a more severe phenotype, with ~20 febrile seizures at age 6 mo–5 years, 10 afebrile tonic-clonic seizures at age 5–15 years, and occasional complex partial seizures associated with mild learning difficulties. She was classified as having FS+ and complex partial seizures. Her older sister, V-15, had typical FS+, but their younger brother, V-17, had no FS but had two afebrile tonic-clonic seizures at ages 12 years 6 mo and 14 years. For purposes of linkage analysis, he was regarded as affected, although he had only afebrile tonic-clonic seizures. All affected subjects were of normal or superior intellect, except V-16 (see above), and all had a normal neurological examination. Electroencephalography (EEG) studies had been performed infrequently during the active phase of the epilepsy, and the results usually either were normal or were reported as showing generalized discharges.

The second Israeli family was of Druze origin; the parents were from different but proximate villages and were not known to be related. This family spans two generations, and four family members had seizures (fig. 1*C*). The proband, I-2, age 41 years, had had hundreds of tonic-clonic seizures, sometimes with fever. These began at age 4 years and continued, at a rate of approximately one per month, until the time of the study. The proband was mildly intellectually impaired. EEG showed generalized irregular spike-wave and polyspike-wave discharges, and FS+ was diagnosed. Of her four children, the oldest, II-1, was unaffected, two, II-2 and II-4, had FS, and one, II-3, had FS+.

Isolation and Sequencing of SCN1A *Genomic Clones*

A human bacterial artificial chromosome (BAC) library from Genome Systems was screened by a PCR product amplified with the primer pair 5′-AGATGACCAGAGTGAATATGTGACTAC-3′ and 5′-CCAATGGTAAAATAATAATGGCGT-3′, designed from the partial cDNA sequence of *SCN1A* (GenBank accession number X65362). BAC 129e04 was selected for subcloning and sequencing. BAC DNA was nebulized and size fractionated on 0.8% agarose gels, and 2–4-kb fragments were subcloned into pUC18 vector. This sublibrary was used in shotgun sequencing to generate single complete coverage of the BAC clone. Sequence data were assembled in contigs, by Phred, Phrap, and Gap4 high-throughput sequencing software. Exon-intron boundaries were predicted on the basis of the rat *Scn1a* cDNA sequence (GenBank accession number M22253) because the full-length human cDNA sequence was not known.

Single-Stranded Conformation Analyses (SSCA) and Sequencing

HEX-labeled primers were designed to amplify all exons of *SCN1A* (table 1). A 30-ng sample of patient DNA was amplified in a total volume of 10 μl. Products were separated on nondenaturing 4% polyacrylamide gels containing 2% glycerol, by the GelScan 2000 (Corbett Research). PCR products showing a conformational change were reamplified, by unlabeled primers from 100 ng of genomic DNA, and were sequenced by the BigDye Terminator ready-reaction kit (PE Biosystems).

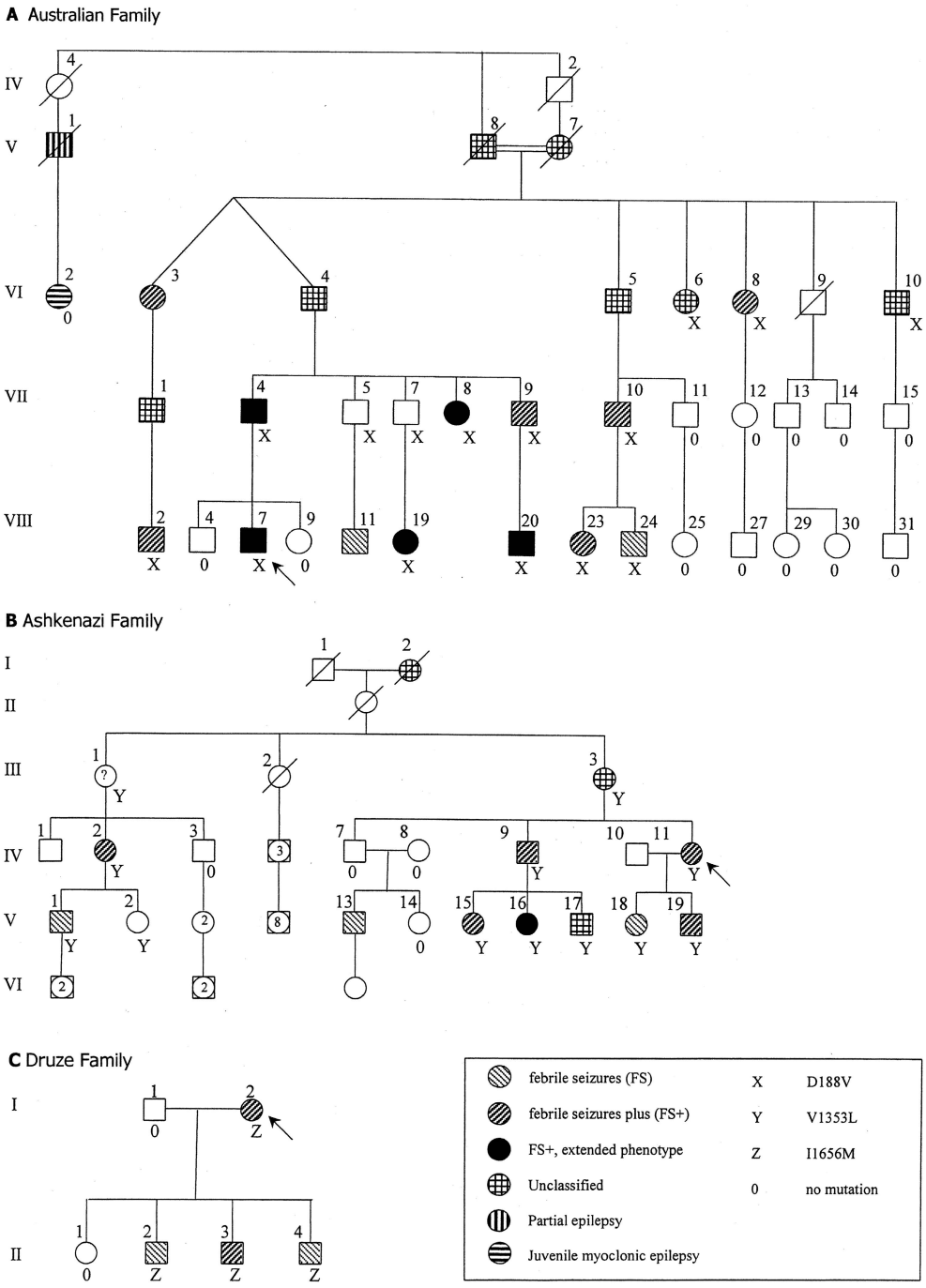


Figure 1 GEFS+ pedigrees. DNA was not available from those individuals not assigned a letter (X, Y, or Z) or a 0. *A*, Australian family, with numbering based on figure 1 in the article by Scheffer and Berkovic (1997). *B*, Ashkenazi family. *C*, Druze family.

Results

The 8,381-bp cDNA of *SCN1A* is organized into 27 exons spanning >100 kb of genomic DNA. To facilitate comparison with related sodium channels *SCN4A*, *SCN5A*, and *SCN8A*, the first untranslated exon is designated "exon 1A," and the second exon, containing the start codon, remains "exon 1." Two different splice-donor sites were identified in exon 11, resulting in two transcripts that differ by 11 amino acids in the first cytoplasmic loop. Intronic primers (table 1), designed to amplify each of the 27 exons of *SCN1A*, were used to screen for mutations in patients with GEFS+.

A total of 53 unrelated patients with GEFS+ were

Table 1

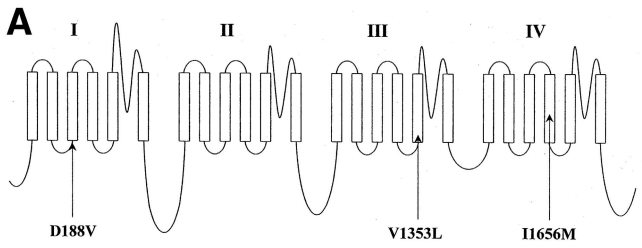
Primers Designed to Amplify All Exons of *SCN1A*

Exon[a]	Primer (5′→3′) Forward	Primer (5′→3′) Reverse	Size (bp)
1A	TACCATAGAGTGAGGCGAGG	ATGGACTTCCTGCTCTGCCC	356
1	CCTCTAGCTCATGTTTCATGAC	TGCAGTAGGCAATTAGCAGC	448
2	CTAATTAAGAAGAGATCCAGTGACAG	GCTATAAAGTGCTTACAGATCATGTAC	356
3	CCCTGAATTTTGGCTAAGCTGCAG	CTACATTAAGACACAGTTTCAAAATCC	263
4	GGGCTACGTTTCATTTGTATG	GCAACCTATTCTTAAAGCATAAGACTG	355
5	AGGCTCTTTGTACCTACAGC	CATGTAGGGTCCGTCTCATT	199
6	CACACGTGTTAAGTCTTCATAGT	AGCCCCTCAAGTATTTATCCT	394
7	GAACCTGACCTTCCTGTTCTC	GTTGGCTGTTATCTTCAGTTTC	241
8	GACTAGGCAATATCATAGCATAG	CTTTCTACTATATTATCATCCGG	320
9	TTGAAAGTTGAAGCCACCAC	CCACCTGCTCTTAGGTACTC	363
10	GCCATGCAAATACTTCAGCCC	CACAACAGTGGTTGATTCAGTTG	480
11a	TGAATGCTGAAATCTCCTTCTAC	CTCAGGTTGCTGTTGCGTCTC	306
11b	GATAACGAGAGCCGTAGAGAT	TCTGTAGAAACACTGGCTGG	315
12	CATGAAATTCACTGTGTCACC	CAGCTCTTGAATTAGACTGTC	347
13a	ATCCTTGGGAGGTTTAGAGT	CATCACAACCAGGTTGACAAC	292
13b	CTGGGACTGTTCTCCATATTG	GCATGAAGGATGGTTGAAAG	277
14	CATTGTGGGAAAATAGCATAAGC	GCTATGCAGAACCCTGATTG	338
15a	TGAGACGGTTAGGGCAGATC	AGAAGTCATTCATGTGCCAGC	348
15b	CTGCAAGATCGCCAGTGATTG	ACATGTGCACAATGTGCAGG	276
16a	GTGGTGTTTCCTTCTCATCAAG	TCTGCTGTATGATTGGACATAC	387
16b	CAACAGTCCTTCATTAGGAAAC	ACCTTCCCACACCTATAGAATC	353
17	CTTGGCAGGCAACTTATTACC	CAAGCTGCACTCCAAATGAAAG	232
18	TGGAAGCAGAGACACTTTATCTAC	GTGCTGTATCACCTTTTCTTAATC	234
19	CCTATTCCAATGAAATGTCATATG	CAAGCTACCTTGAACAGAGAC	318
20	CTACACATTGAATGATGATTCTGT	GCTATATACAATACTTCAGGTTCT	216
21a	ACCAGAGATTACTAGGGGAAT	CCATCGAGCAGTCTCATTTCT	303
21b	ACAACTGGTGACAGGTTTGAC	CTGGGCTCATAAACTTGTACTAAC	297
22	ACTGTCTTGGTCCAAAATCTG	TTCGATTAATTTTACCACCTGATC	267
23	AGCACCAGTGACATTTCCAAC	GGCAGAGAAAACACTCCAAGG	272
24	GACACAGTTTTAACCAGTTTG	TGTGAGACAAGCATGCAAGTT	207
25	CAGGGCCAATGACTACTTTGC	CTGATTGCTGGGATGATCTTGAATC	477
26-1	CGCATGATTTCTTCACTGGTTGG	GCGTAGATGAACATGACTAGG	247
26-2	TCCTGCGTTGTTTAACATCGG	ATTCCAACAGATGGGTTCCCA	288
26-3	TGGAAGCTCAGTTAAGGGAGA	AGCGCAGCTGCAAACTGAGAT	261
26-4	CCGATGCAACTCAGTTCATGGA	GTAGTGATTGGCTGATAGGAG	274
26-5	AGAGCGATTCATGGCTTCCAATCC	TGCCTTCTTGCTCATGTTTTTCCACA	335
26-6	CCTATGACCGGGTGACAAAGCC	TGCTGACAAGGGGTCACTGTCT	242

[a] More than one PCR was required to amplify the large exons 11, 13, 15, 16, 21, and 26.

screened by fluorescent SSCA, including two families consistent with mapping to the same location as *SCN1A* on chromosome 2 (fig. 1*A* and *B*). No mutations were found in the 17 sporadic cases of GEFS+ that were tested. Of the 36 families tested, 3 were found to have point mutations in *SCN1A*, which alter the amino acid sequence and are not present in the control population ($n = 60$). The phenotype in the family in fig. 1*A* previously had been mapped to chromosome 2 (Lopes-Cendes et al. 2000) and carries a c.563A→T mutation that segregates with affected family members. This mutation in exon 4 of *SCN1A* results in a D188V amino acid substitution that lies just outside the S3 segment of domain I (fig. 2*A*). The aspartic acid residue is conserved in all identified sodium channels in humans, as well as in many different animal species—except the jellyfish, which has an arginine at this residue, and the flatworm, which has a serine (fig. 2*B*). (The published rat *Scn2a* sequence [GenBank accession number NM_012647] also has an arginine in place of the aspartic acid, at residue 188.)

A mutation in exon 21 (c.4057G→C) of *SCN1A* was found to segregate with GEFS+ in the Ashkenazi family (fig. 1*B*). This mutation changes a highly conserved amino acid (V1353L) located in the S5 segment of domain III (fig. 2). One family member, V-13, did not carry the mutation (fig. 1*B*). This was determined by testing the DNA of a parent of this family member, since the subject's DNA was unavailable. This individual, who had FS that terminated at an early age, is likely to be



B Sodium Channel Amino Acid Alignment

i) D188V

SCN1A	F	T	F	L	R	D	P	W	N	W	L
RAT SCN1A	-	-	-	-	-	-	-	-	-	-	-
SCN2A	-	-	-	-	-	-	-	-	-	-	-
SCN3A	-	-	-	-	-	-	-	-	-	-	-
SCN4A	-	-	-	-	-	-	-	-	-	-	-
SCN5A	-	-	-	-	-	-	-	-	-	-	-
SCN6A	-	S	-	-	G	-	-	-	-	-	-
SCN8A	-	-	-	-	-	-	-	-	-	-	-
SCN9A	-	-	-	-	-	-	-	-	-	-	-
SCN10A	-	-	Y	-	-	-	-	-	-	-	-
SCN11A	-	S	-	-	-	-	-	-	-	-	-
SCN12A	-	S	-	-	-	-	-	-	-	-	-
EL. EEL	-	-	-	-	-	-	-	-	-	-	-
DROS	-	-	Y	-	-	-	A	-	-	-	-
SQUID	-	-	Y	-	-	-	A	-	-	-	-
FLATWORM	-	-	Y	-	-	S	I	-	-	-	-
JELLYFISH	Y	S	Y	-	-	N	S	-	-	-	-

ii) V1353L

SCN1A	M	N	V	L	L	V	C	L	I	F	W
RAT SCN1A	-	-	-	-	-	-	-	-	-	-	-
SCN2A	-	-	-	-	-	-	-	-	-	-	-
SCN3A	-	-	-	-	-	-	-	-	-	-	-
SCN4A	-	-	-	-	-	-	-	-	-	-	-
SCN5A	-	-	-	-	-	-	-	-	-	-	-
SCN6A	L	-	-	F	-	-	-	-	M	I	-
SCN8A	-	-	-	-	-	-	-	-	-	-	-
SCN9A	-	-	-	-	-	-	-	-	-	-	-
SCN10A	-	-	-	-	-	-	-	-	-	-	-
SCN11A	L	-	-	-	-	-	-	-	-	-	-
SCN12A	L	-	-	-	-	-	-	-	-	-	-
EL. EEL	-	-	-	-	-	-	-	-	-	-	-
DROS	F	-	-	-	-	-	-	-	-	-	-
SQUID	F	-	-	-	-	-	-	-	V	-	-
FLATWORM	F	-	-	M	V	-	-	-	V	-	-
JELLYFISH	A	-	-	-	-	-	-	G	V	-	-

iii) I1656M

SCN1A	K	G	A	K	G	I	R	T	L	L	F
RAT SCN1A	-	-	-	-	-	-	-	-	-	-	-
SCN2A	-	-	-	-	-	-	-	-	-	-	-
SCN3A	-	-	-	-	-	-	-	-	-	-	-
SCN4A	R	-	-	-	-	-	-	-	-	-	-
SCN5A	R	-	-	-	-	-	-	-	-	-	-
SCN6A	-	-	P	-	V	F	H	N	-	M	L
SCN8A	-	-	-	-	-	-	-	-	-	-	-
SCN9A	-	-	-	-	-	-	-	-	-	-	-
SCN10A	R	A	-	-	-	-	-	-	-	-	-
SCN11A	R	A	-	-	-	-	-	-	-	-	-
SCN12A	R	A	-	-	-	-	-	-	-	-	-
EL. EEL	-	-	-	-	-	-	-	-	-	-	-
DROS	-	-	-	-	-	-	-	-	-	-	-
SQUID	-	S	-	-	-	-	-	-	-	-	-
FLATWORM	-	S	-	R	-	-	-	-	-	-	-
JELLYFISH	D	-	-	-	-	-	-	Q	-	-	-

Figure 2 *SCN1A* mutations. *A*, Schematic of *SCN1A*, showing positions of the three mutations identified in this study. *B*, Sodium-channel amino acid alignments, showing alignment of sodium-channel amino acids surrounding the three *SCN1A* mutations.

a phenocopy. A third mutation (c.4968C→G), discovered in the Druze family (fig. 1C), changes an amino acid (I1656M) in the S4 segment of domain IV (fig. 2).

During the mutation screen of *SCN1A*, several single-nucleotide polymorphisms (SNPs) were identified (table 2). The R1928G variant was found at low frequency in both GEFS+ and control populations. The T1067A variant was common in both populations. The remaining SNPs identified did not alter the amino acid sequence of SCN1A (table 2). The 53 patients with GEFS+ were also screened for mutations in *SCN1B*. Two families had the same mutation—C121W, due to a c.387C→G mutation in exon 3—as was seen in the family reported by Wallace et al. (1998). Details will be presented elsewhere.

Discussion

The complete genomic structure of *SCN1A* has been determined to enable mutation screening in families with GEFS+. This gene shows high homology to *SCN2A* and *SCN3A*, at both the DNA and the protein levels. The close proximity of all three of these genes to each other on chromosome 2 indicates likely duplication events during the evolution of the sodium-channel–gene family. Inspection of the splice junctions shows that there is close agreement with consensus splice motifs, for all introns bounded by GT-AG—except for introns 2 and 23, which exhibit deviation from the consensus splice pattern and are bounded by AT-AC terminal dinucleotides. These rare splice-site variations are conserved in other characterized sodium-channel subunits (e.g., *SCN4A*, *SCN8A*, and the more distantly related *SCN5A*), indicating their ancient origin. The intron positions also are highly conserved, with most variation seen in the region that codes for the cytoplasmic loop between domains I and II of the gene. Within this region, an alternative splicing of exon 11 of *SCN1A*, comparable to the alternative splicing of exon 10B in *SCN8A*, was found (Plummer et al. 1998). Cytoplasmic loop 1 varies in both

Table 2

Sequence Variants of *SCN1A* Detected in 53 GEFS+ Samples and in 60 Normal Samples

	SCN1A Polymorphism		Frequency (%)	
Position	Mutation	Amino Acid Change	GEFS+	Normal
Intron 13	IVS13−37C→A	...	2.4	8.6
Exon 14	c.2522C→G	...	2.4	8.6
Intron 15	IVS15+54A→G	...	36.3	23.6
Exon 15	c.2889T→C	...	1.2	.0
Exon 16	c.3199A→G	T1067A	29.5	30.8
Exon 26	c.5782C→G	R1928G	1.2	1.7

length and composition and is the proposed site of functional diversity among different sodium channels (Plummer and Meisler 1999).

Determination of the genomic structure of *SCN1A,* which was in progress when the first *SCN1A* mutations were reported (Escayg et al. 2000), facilitated the design of primers for mutation screening of patients with GEFS+. GEFS+ has been shown to be due to mutations in two sodium-channel subunits (Wallace et al. 1998; Escayg et al. 2000). Among the 53 probands with GEFS+, three new *SCN1A* mutations were identified; these were in three families of different ethnic backgrounds and segregated with the GEFS+ phenotypes. One of the mutations, I1656M, is similar to those described by Escayg et al. (2000), in that it lies within the S4 segment of the transmembrane domain. The S4 segment has a role in channel gating and mutations in this region of *SCN1A* reduce the rate of inactivation (Kühn and Greeff 1996). The V1353L mutation is within the S5 segment of transmembrane domain III, in a highly conserved region. Mutations in the S5 segments of *SCN4A* that cause hyperkalemic periodic paralysis have been shown also to affect the rate of channel inactivation (Bendahhou et al. 1999). The D188V mutation lies just outside the S3 segment of transmembrane domain I, in another highly conserved region of *SCN1A*. This mutation was not detected in 120 control chromosomes and segregates with the affected members of the family. It is possible that D188V is a rare variant that is in linkage disequilibrium with the true mutation. The entire coding region of *SCN1A* was sequenced, and no other mutations were found. Further studies will be necessary to determine the effect that D188V has on sodium-channel function.

The *SCN1A* mutations that cause GEFS+ are in different functional domains of the channel, but they all may lead to decreased inactivation resulting in a persistent depolarizing sodium current and hyperexcitability. The mutations in the muscle sodium-channel *SCN4A* that cause skeletal muscle myotonias and paralysis are similar in that they are found along the length of the gene. In general, these mutations disable inactivation of the sodium channel, producing either repetitive action-potential firing (myotonia) or electrical silence (paralysis) (Cannon 2000). Similarly, mutations in different regions of the cardiac sodium channel *SCN5A* lead to repetitive electrical activity in the heart, owing to altered channel inactivation (Bennett et al. 1995; Wang et al. 1996). As further sodium-channel mutations are identified, our understanding of the functionally important domains of this class of ion channel will increase.

There are now five families known to have *SCN1A* mutations (Escayg et al. 2000; present study) and three families known to have *SCN1B* mutations (Wallace et al. 1998; R.H.W., unpublished data). The genetic heterogeneity of GEFS+ is now well established. The present study extends the distribution of *SCN1A* mutations to populations of Middle Eastern origin. No *SCN1A* or *SCN1B* mutations were found in 17 isolated cases of GEFS+. Of the 36 familial cases studied, 3 had mutations in *SCN1A,* and 3 had mutations in *SCN1B*. Together, *SCN1A* and *SCN1B* account for ~17% (6/36) of cases of familial GEFS+. Some mutations may have been missed either because they occur outside the gene regions screened or because the detection rate of the fluorescent SSCA screening method is <100%. Even so, the proportion of GEFS+ caused by these two sodium channels is still likely to be low, indicating that other genes are involved; obvious candidates include other neuronal sodium channels and proteins that interact with sodium channels.

Acknowledgments

This project was funded by the National Health and Medical Research Council of Australia, National Institute of Health grant NS 32387 to A.L.G., the Women's and Children's Hospital Research Foundation, Bionomics Limited, and the Epilepsy Foundation, through the generosity of the Kathy Holden Genetic Research Fund, and by a grant from the Roland and Ruby Holden Foundation, on behalf of Ronald and Arlene Holden. The Israeli families were characterized while S.F.B. was on sabbatical leave at the Department of Neurology, Tel Aviv Sourasky Medical Centre. S.F.B. was sponsored by Prof. Amos Korczyn, Sieratzki Chair in Neurology, Tel Aviv University. We also thank the families for their participation.

Electronic-Database Information

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

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for partial cDNA sequence of human *SCN1A* [accession number X65362], rat *Scn1a* cDNA sequence [accession number M22253], and arginine in the rat *Scn2A* sequence [accession number NM_012647])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for GEFS+ [MIM 604236])

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