Am. J. Hum. Genet. 68:866–873, 2001

A Novel *SCN1A* Mutation Associated with Generalized Epilepsy with Febrile Seizures Plus—and Prevalence of Variants in Patients with Epilepsy

Andrew Escayg,[1,*] Armin Heils,[2,*] Bryan T. MacDonald,[1] Karsten Haug,[3] Thomas Sander,[4] and Miriam H. Meisler[1]

[1]Department of Human Genetics, University of Michigan, Ann Arbor; [2]University Clinic of Epileptology, and [3]Department of Human Genetics, University of Bonn, Bonn; and [4]Department of Neurology, University Hospital Charité, Campus Virchow Clinic, Humboldt University of Berlin, Berlin

We recently described mutations of the neuronal sodium-channel α-subunit gene, *SCN1A*, on chromosome 2q24 in two families with generalized epilepsy with febrile seizures plus (GEFS+) type 2. To assess the contribution that *SCN1A* makes to other types of epilepsy, 226 patients with either juvenile myoclonic epilepsy, absence epilepsy, or febrile convulsions were screened by conformation-sensitive gel electrophoresis and manual sequencing of variants; the sample included 165 probands from multiplex families and 61 sporadic cases. The novel mutation W1204R was identified in a family with GEFS+. Seven other coding changes were observed; three of these are potential disease-causing mutations. Two common haplotypes, with frequencies of .67 and .33, were defined by five single-nucleotide polymorphisms (SNPs) spanning a 14-kb region of linkage disequilibrium. An SNP located 18 bp upstream of the splice-acceptor site for exon 3 was observed in 7 of the 226 patients but was not present in 185 controls, suggesting possible association with a disease mutation. This work has confirmed the role of *SCN1A* in GEFS+, by identification of a novel mutation in a previously undescribed family. Although a few candidate disease alleles were identified, the patient survey suggests that *SCN1A* is not a major contributor to idiopathic generalized epilepsy. The *SCN1A* haplotypes and SNPs identified here will be useful in future association and linkage studies.

Introduction

Recent progress in the positional cloning of genes responsible for monogenic epilepsies has demonstrated the involvement of ion-channel genes in several rare disorders (McNamara 1999). Sodium-channel mutations have been demonstrated for the epilepsy syndrome GEFS+ (generalized epilepsy with febrile seizures plus) (MIM 604236 and MIM 604233). Individuals with GEFS+ are characterized by febrile seizures that persist beyond age 6 years and by heterogeneous afebrile seizures that may include tonic-clonic, myoclonic, atonic, and absence seizures (Scheffer and Berkovic 1997). A mutation in the voltage-gated sodium-channel β-subunit *SCN1B* was identified in 1998 in a family with GEFS+ type 1 linked to chromosome 19p13 (Wallace et al. 1998). We described mutations of the neuronal sodium-channel α-subunit *SCN1A* in two families with GEFS+ type 2 linked to chromosome 2q24 (Escayg et al. 2000*b*). In the mouse, mutation of the related sodium-channel *SCN2A* also results in a dominantly inherited, progressive seizure disorder (Kearney et al. 2001).

SCN1A is one of the four voltage-gated sodium channels that are widely expressed in neurons of the CNS and peripheral nervous system (PNS) (Plummer and Meisler 1999). The differences in firing patterns and excitability of different classes of neurons are influenced by their specific complement of voltage-gated sodium, potassium, and calcium channels, as well as by the extent of posttranslational modifications of the channel proteins. Subtle differences in channel properties can have significant physiological effects and may underlie individual variation in neurological function (Kohrman et al. 1996; Bulman 1997).

The observation that patients with GEFS+ experience several types of seizures has suggested that genetic variation in *SCN1A* could contribute to other common forms of idiopathic generalized epilepsy. To test this possibility, we screened 226 patients with either juvenile myoclonic epilepsy (JME), childhood or juvenile absence epilepsy (CAE and JAE, respectively), febrile convulsions (FC), or generalized tonic-clonic seizures (GTCS). Twenty *SCN1A* variants were identified, including 8 amino acid substitutions and 12 noncoding variants. One amino acid substitution, W1204R, cosegregated with GEFS+ in a previously undescribed family, making this the third

Received January 11, 2001; accepted for publication February 16, 2001; electronically published March 14, 2001.

Address for correspondence and reprints: Dr. Miriam H. Meisler, Department of Human Genetics, University of Michigan, 4708 Medical Science II, Ann Arbor, MI 48109-0618. E-mail: meislerm@umich.edu

* The first two authors contributed equally to this work.

0002-9297/2001/6804-0009$02.00

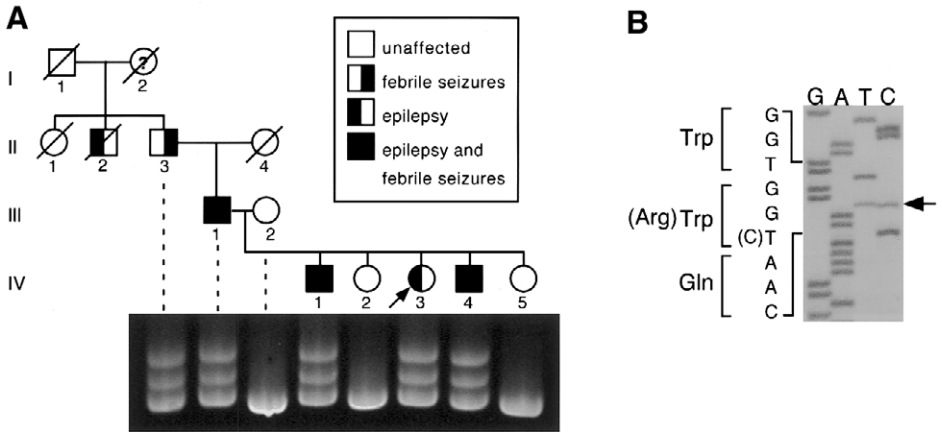

Figure 1 Mutation W1204R, cosegregating with disease in a family with GEFS+. *A*, Family with GEFS+, with 13 individuals from four generations. Six individuals were classified as affected. DNA was obtained from five affected and three unaffected family members. CSGE analysis of exon 18 of *SCN1A* identified a unique pattern in the proband (*arrow*). The same pattern was observed in the other affected individuals. *B*, Sequence analysis revealing a T→C transition (*arrow*) resulting in amino acid substitution W1204R.

SCN1A mutation associated with GEFS+. Several variants identified in sporadic cases require further testing as possible susceptibility factors.

Subjects and Methods

Subjects

DNA was obtained from index patients from 165 unrelated families with idiopathic generalized epilepsy (Haug et al. 2000; Sander et al. 2000). These patients included 72 with JME, 83 patients with either CAE or JAE, 4 with random GTCS on awakening, and 6 with a history of FC, who had at least one first-degree relative who was affected. Sixty-one sporadic cases of idiopathic generalized epilepsy (IGE) were also included in the study. Diagnostic classification followed the proposal of the Commission on Classification and Terminology of the International League Against Epilepsy (1989). The diagnosis of JME was based on repetitive myoclonic jerks, in arms and shoulders, on awakening. Diagnostic criteria for idiopathic absence epilepsy were a brief loss of consciousness with pyknoleptic daily clusters of absence seizures. The diagnosis for FC followed the proposal of the National Institutes of Health Consensus Development Panel (Consensus Statement 1980). Electroencephalography (EEG) recordings were obtained from most patients and demonstrated a normal background activity. Paroxysmal generalized spike-and-wave discharges were present in the patients with IGE. Each *SCN1A* variant observed in patients was assayed in 96–268 ethnically matched, unrelated controls who had been described elsewhere (Haug et al. 2000; Sander et al. 2000). Written consent was obtained from all participants, and the study was approved by the institutional ethics committees.

Mutation Detection

The 26 exons of *SCN1A* were individually amplified from genomic DNA in 28 PCR reactions using primers based on intronic sequence (Escayg et al. 2000*b*). PCR amplification was performed at 32 cycles of 1 min at 94°C, 45 s at 60°C, and 1 min at 72°C. PCR products were incubated at 99°C for 10 min, followed by 35 min at 68°C to facilitate heteroduplex formation, and were analyzed by conformation-sensitive gel electrophoresis (CSGE) with visualization by ethidium bromide staining as described elsewhere (Escayg et al. 2000*a*). PCR products with mobility variants were gel purified by means of the Qiaex II gel extraction kit (Qiagen) and were manually sequenced by means of the ThermoSequenase sequencing kit (USB).

Statistical Analysis

Allele and genotype frequencies in the patients and in the control groups were compared by either χ^2 analysis or Fisher's exact test. A two-tailed type I error rate of 5% was chosen for the analyses. A correction for multiple testing was performed according to the Bonferroni procedure.

Results

W1204R: Cosegregation with GEFS+

Multiple members of a previously undescribed family from Germany had a history of febrile and afebrile seizures consistent with the classification of GEFS+ (Schef-

Table 1

Clinical Features of Affected Individuals from the Family with GEFS+

Affected Individual	Age (years)		Type of Afebrile Seizure	Clinical Results	
	At Onset of FC	At Last FC		EEG	MRI
II-2	None	...	Unknown	Not available	Not available
II-3	.5	5	None	Not available	Not available
III-1	1	8	GTCS	Normal	Not available
IV-1	2	7	Myoclonic-astatic	Sharp slow waves, spike waves, polyspike wave	Normal
IV-3	None	...	Myoclonic	Polyspike wave	Normal
IV-4	1	6	GTCS	Irregular spike waves	Normal

Note.—The family pedigree is shown in figure 1.

fer and Berkovic 1997). The four-generation pedigree is presented in figure 1. Six individuals in this family were classified as affected (table 1). Individuals III-1, IV-1, and IV-4 reported a history of childhood febrile and afebrile seizures. Individual II-3 had febrile seizures until age 5 years but did not have afebrile seizures. The proband (IV-3) was diagnosed with severe myoclonic seizures and did not have a history of febrile seizures. A detailed family history indicated that individual II-2 had epilepsy as an adult. The disease status of individual I-2 is unclear. EEG recordings and results of magnetic-resonance imaging (MRI) were obtained for selected individuals (table 1). Normal EEG patterns were recorded from the unaffected individuals: III-2, IV-2, and IV-5. Results of neurological examination were normal in all individuals except IV-1, who had mild intellectual disability and severe seizures that resulted in permanent institutional care. The clinical features of the affected members of this family are summarized in table 1.

DNA samples from the eight living family members were screened for *SCN1A* mutations. The abnormal CSGE conformer of exon 18 was observed in the proband and in the other four affected individuals (fig. 1*A*). Sequence analysis of the PCR product revealed a T→C transition that was responsible for the amino acid substitution W1204R (fig. 1*B*). The three unaffected family members did not carry the mutation. This nonconservative amino acid substitution was not observed in 268 unaffected controls (536 chromosomes). W1204 is located in cytoplasmic loop 2, within 10 amino acids of the transmembrane segment D3S1 (fig. 2). The functional importance of this residue is demonstrated by evolutionary conservation in 12 of 13 mammalian, vertebrate, and invertebrate sodium channels (fig. 3).

Mutation Screening

Two hundred twenty-five additional patients were screened by exon amplification and CSGE. Nineteen additional variants were detected, including 18 single-nucleotide polymorphisms (SNPs) and 1 dinucleotide deletion (table 2); 7 of these SNPs encoded novel amino acid substitutions. Allele frequencies for six coding SNPs (cSNPs) were <1% and did not differ significantly be-

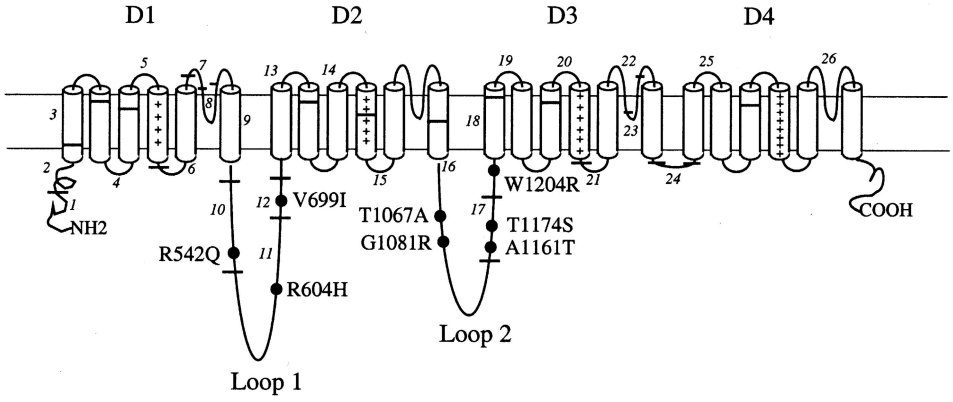

Figure 2 Amino acid substitutions in *SCN1A* that are identified in the present study. The sodium channel is composed of four internally homologous domains, D1–D4, each containing six transmembrane segments. The eight amino acid substitutions identified in the patient population are located in cytoplasmic loops 1 and 2. The only change that affects an evolutionarily conserved residue is W1204, located close to the cytoplasmic surface of transmembrane domain D3. Exon/intron boundaries are marked by horizontal bars; exon designations are in italics.

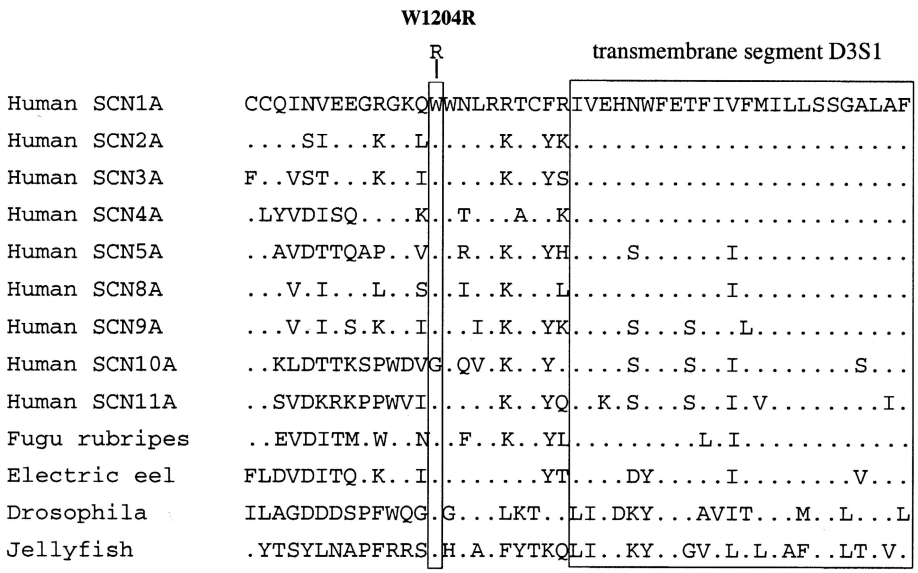

Figure 3 Evolutionary conservation of tryptophan residue 1204. W1204 is located 10 residues upstream of transmembrane segment D3S1. This residue is highly conserved in vertebrate and invertebrate channels, including all of the other members of the mammalian gene family. The *SCN1A* sequence is that reported by Escayg et al. (2000*b*); GenBank accession numbers, from top to bottom, are M94055, AJ251507, M81758, M77235, AB027567.1, X82835, AF117907.1, AF188679, D37977, M22252, M32078, and AF047380. Electric eel = *Electrophorus electricus;* Drosophila = *D. melanogaster;* jellyfish = *Polyorchis penicillatus.*

tween patient and control populations ($P > .05$, by Fisher's exact test). One coding variant, T1067A, was polymorphic in patients and controls, with an allele frequency of .33 in both groups.

Three coding variants identified in familial cases did not cosegregate with the disease. V699I in exon 12 was identified in the proband of a family with JAE and FC. Other affected family members did not carry the mutation. R604H in exon 11 was identified in two probands from unrelated families with JME. The mutant allele in one of the two families carries the substitution A1161T, in exon 17, which cosegregates with R604H (table 2). R604H disrupts a protein kinase A (PKA) consensus site that is conserved in the four major sodium channels of the CNS (fig. 4*A*). Analysis of DNA from additional family members demonstrated multiple recombinations with the disease, including affected individuals in both families who did not carry the mutation (data not shown).

Three cSNPs were identified in sporadic cases of epilepsy for which family records were not available. R542Q was found in a patient with JME and is located in a consensus tyrosine kinase phosphorylation site (fig. 4*B*). G1081R and T1174S were identified in patients with JAE and JME, respectively, and T1174S was also observed in one control (table 2). Since family members were not available for analysis, the role that these amino acid substitutions play in seizure susceptibility could not be evaluated and warrants further analysis. The relationship between the eight coding variants and disease status is summarized in table 3.

Noncoding SNPs

Seven noncoding SNPs were present at polymorphic frequencies (i.e., >1%) (table 2), and five were present at lower frequencies. Allele frequencies did not differ significantly between the 226 patients and 96–194 ethnically matched controls, with one exception: the SNP in intron 2 appeared to be more frequent in patients (7/226) than in controls (0/185) ($P = .019$, by Fisher's exact test [two tailed]), but the difference between the patients and controls was not significant after correction for multiple testing. The patients with this variant were diagnosed as having JAE (4 cases), JME (2 cases), or CAE (1 case). Additional studies, in independent samples, will be required in order to determine whether this SNP is associated with predisposition to epilepsy.

SCN1A *Haplotypes*

Two major haplotypes are defined by the consistent association of five SNPs in *SCN1A* in 322 unrelated individuals, including patients and controls (table 4). Haplotypes 1 and 2 exhibit frequencies of .67 and .33 in this northern-European population. The genotype frequencies are in agreement with Hardy-Weinberg expectations. The haplotypes were confirmed by cosegregation in meiosis (data not shown). Minor haplotypes are de-

Table 2

Single-Nucleotide Substitutions in Human *SCN1A*

Location	cDNA Nucleotide[a] (Amino Acid Change)	Major/Minor SNP Alleles	No. (Frequency) of Minor Allele in 226 Patients	No. (Frequency) of Minor Allele in 96 Controls[b]	Haplotype of Minor Allele[c]
Intron 2	384−20	G/C	7 (.02)	0 [185]	2
Intron 6	965−21	T/C	151 (.33)	64 (.33)	2
Intron 7	1028+21	C/T	…[d]	…	…
Exon 8	1131	A/C	2 (.004)	4 (.02) [194]	1
Intron 8	1171−9,10	TT/ΔTT	16 (.04)	7 (.04)	1
Exon 9	1212	G/A	151 (.33)	64 (.33)	2
Exon 10	1625 (R542Q)	G/A	1 (.002)	0	1
Exon 11	1811 (R604H)	G/A	2 (.004)	0	1
Exon 12	2095 (V699I)	G/A	1 (.002)	0	1
Exon 13	2292	C/T	151 (.33)	64 (.33)	2
Intron 13	2416−37	C/A	151 (.33)	64 (.33)	2
Intron 13	2416−72	A/G	…[d]	…	…
Exon 16	3199 (T1067A)	A/G	151 (.33)	64 (.33)	2
Exon 16	3241 (G1081R)	G/A	1 (.002)	0	…
Exon 17	3481 (A1161T)	G/A	1 (.002)	0	1
Exon 17	3521 (T1174S)	C/G	1 (.002)	1 (.005)	…
Exon 18	3610 (W1204R)	T/C	1 (.002)	0 [268]	1
Intron 23	4476+33	G/A	4 (.009)	2 (.01)	2
Exon 25	4731	T/C	1 (.002)	1 (.005)	…
Exon 26	5418	G/A	8 (.02)	3 (.005) [183]	2

[a] Nucleotide position is that of human *SCN1A* cDNA (Escayg et al. 2000*b*).
[b] In those cases in which >96 controls were tested, the total number is shown in square brackets.
[c] Haplotypes are as in table 4.
[d] Identified by sequencing a small number of individuals.

fined by the rare SNPs, which are all restricted to either haplotype 1 or haplotype 2 (table 2).

The SNPs defining the haplotypes span a 14-kb region of linkage disequilibrium (LD) in *SCN1A* (fig. 5). The positions of related sodium-channel genes in the cluster on chromosome 2q24 are shown on the clone contig, which was generated by BLAST alignment of cDNA and genomic sequences in the GenBank high-throughput genomic-sequence database. In the cluster, *SCN1A* and *SCN2A* are orientated tail-to-tail, with their C-terminal coding exons separated by 600 kb. *SCN2A* and *SCN3A* are orientated head-to-head separated by 100 kb that could contain shared regulatory sequences. The PNS-channel *SCN9A* is located 120 kb distal to *SCN1A*. The microsatellite *D2S2330*, located between *SCN1A* and *SCN2A*, is closely linked to the inherited epilepsy locus FEB3 (Peiffer et al. 1999), indicating that *SCN1A* and *SCN2A* are strong candidates genes for this disorder.

Discussion

This is the first systematic study of population variation in a neuronal sodium-channel α-subunit gene. We have identified 20 variants of *SCN1A*, including 8 amino acid substitutions, in 226 patients with IGE subtypes and FC. The amino acid substitution W1204R has been found

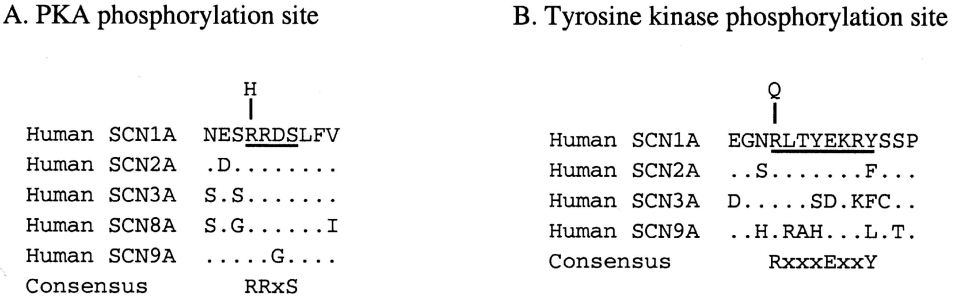

Figure 4 *SCN1A* variants R604H and R542Q, which disrupt potential sites of protein phosphorylation. *A*, Consensus protein kinase A site in exon 11 (*underlined*), which is conserved in several sodium channels and is disrupted by the substitution R604H. *B*, Consensus tyrosine kinase phosphorylation site in exon 10 (*underlined*), which is present only in *SCN1A* and is disrupted by the substitution R542Q.

Table 3

Coding Variation in *SCN1A*, and Relationship to Disease

Substitution	Domain	Type of Epilepsy	Genetic Observation(s)
W1204R	Loop 2	GEFS+ type 2	Cosegregates with disease
R604H	Loop 1	Familial JME (two families)	Discordant; absent in several affected family members
V699I	Loop 1	Familial JAE with FC	Discordant; absent in affected parent and affected sib
A1161T	Loop 2	Familial JME	Discordant; absent in several affected family members
R542Q	Loop 1	Sporadic JME	Family not available
G1081R	Loop 2	Sporadic JAE	Family not available
T1174S	Loop 2	Sporadic JME	Family not available
T1067A	Loop 2	All	Polymorphic in patients and controls

to cosegregate with GEFS+, making this the third *SCN1A* mutation associated with this disease (Escayg et al. 2000*b*). It is interesting that the proband in this family had JME, without any history of febrile seizures or other seizure types. Four other variants identified in this study are potentially associated with disease. The difference, in frequency of the SNP in intron 2, between patients (7/226) and controls (0/185) approached significance, and follow-up studies to detect possible disease association with this variant are warranted. The three amino acid substitutions identified in sporadic cases of epilepsy could also be predisposing mutations and will require further analysis.

The 226 patients were screened for all 26 exons of *SCN1A*, and variants were found in 15 exons (table 2). The 96 controls were then screened for these 15 exons only. No novel variants were found in the controls. Rare coding variants of *SCN1A* were present in 8/226 patients and 1/96 controls. As more data accumulate, it will be interesting to determine whether rare coding variants of sodium channels are consistently more prevalent in patient populations.

All of the coding variants identified in this study are located in the cytoplasmic loops of the sodium-channel protein (fig. 2). The voltage-gated sodium channels contain four domains (D1–D4), each with six transmembrane segments. The transmembrane segments are among the most highly conserved protein domains known. The large cytoplasmic loops are less well conserved among different members of the sodium-channel family, but they are well conserved among mammalian orthologues. The two cytoplasmic loops of human *SCN1A* exhibit amino acid sequence identity of 98% (330/337) and 96% (213/221) with *SCN1A* from rat or mouse, suggesting functional constraints throughout these domains. In the cytoplasmic loops, the only functional motifs that have been recognized thus far are phosphorylation sites (Smith and Goldin 1997; Ratcliffe et al. 2000). Phosphorylation of five protein kinase A sites in loop 1 of *SCN2A* can attenuate sodium current amplitude (Smith and Goldin 1997). The PKA site corresponding to R604H had a minor effect on current amplitude in a previous study (Smith and Goldin 1997) and did not cosegregate with the seizure disorder in two families with JME. Sodium channels may be modulated by tyrosine phosphorylation (Ratcliffe et al. 2000), and the variant R542Q disrupts a predicted tyrosine kinase site in exon 10 of *SCN1A*. Another possible function of the cytoplasmic loops is channel clustering and subcellular localization (Sampo et al. 2000), but specific residues involved in these processes have not been identified.

The amino acid substitution T1067A was observed at a frequency of .33 both in patients and in controls and is clearly a nonpathogenic polymorphism. The substitutions G1081R and T1174S were observed at very low frequency in sporadic cases, and T1174S was also present in one control individual. Understanding the significance of the coding variants in the cytoplasmic loops will depend on further elucidation of the functions of these domains.

The two previously identified *SCN1A* mutations in GEFS+ were located in the voltage-responsive S4 segments (Escayg et al. 2000*b*). The GEFS+ mutation described here, W1204R, is located in the second cyto-

Table 4

Two Major *SCN1A* Haplotypes

Haplotype (No. [Frequency])	SNP at				
	Intron 6: 965-21	Exon 9: 1212	Exon 13: 2292	Intron 13: 2416−37	Exon 16: 3199
1 (429 [.67])	T	G	C	C	A
2 (215 [.33])	C	A	T	A	G

Note.—Data are for 322 unrelated individuals. Genotype frequencies were consistent with Hardy-Weinberg equilibrium.

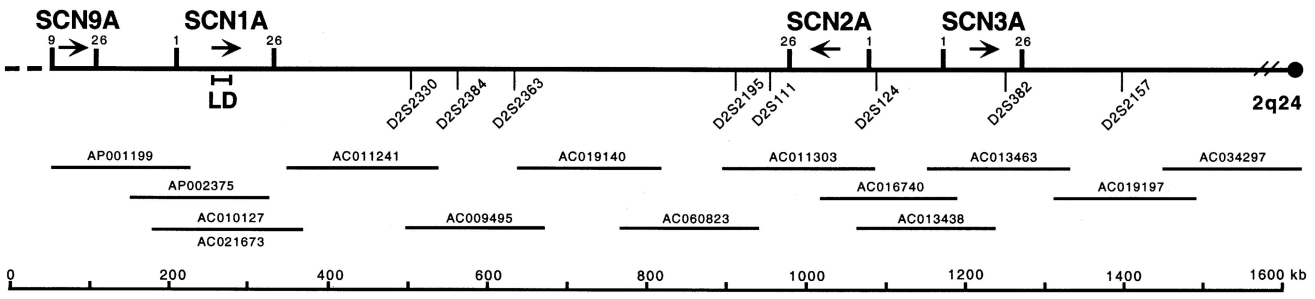

Figure 5 Physical map of sodium-channel gene cluster on chromosome 2q24. Arrows indicate direction of transcription of sodium-channel genes. The positions of polymorphic microsatellites are indicated. BLAST analysis of the GenBank high-throughput genomic-sequence database was used to construct the 1.6-Mb contig containing *SCN1A, SCN2A, SCN3A,* and *SCN9A*. The GenBank accession number of each bacterial artificial chromosome is indicated. LD = 14-kb region of LD in *SCN1A*.

plasmic loop, 10 residues distant from the cytoplasmic surface of the transmembrane segment D3S1. The evolutionary conservation of W1204 is strong evidence for an important function for this residue. Many disease mutations of the muscle and cardiac sodium channels *SCN4A* and *SCN5A* are located in similar sites near the cytoplasmic surfaces of transmembrane segments (Bulman 1997). There are evidently functional constraints on other residues, between W1204 and the start of D3S1, since they are highly conserved in the 11 vertebrate and 2 invertebrate sodium channels compared in figure 3. The number of channels with identical amino acids are as follows: W1204, 12/13; W1205, 11/13; R1208, 13/13;, R/K1209, 13/13; and T1210, 10/13. Functional analysis of the W1204R mutation in *Xenopus* oocytes and mammalian cells will be important for understanding its effect on channel activity.

Four GEFS+ mutations are now known—three in *SCN1A* and one in the β-subunit *SCN1B*. If the *SCN1B* mutation is a loss-of-function mutation, as has been reported elsewhere (Wallace et al. 1998), it would result in slower inactivation of the associated α-subunit, leading to increased persistent current and neuronal hyperexcitability. Persistent sodium currents are thought to underlie ictal epileptiform activity in the brain, and *SCN1A* is expressed in the cell bodies of many neurons that generate persistent currents. Thus, a common electrophysiological mechanism may underlie epilepsies caused by mutations in *SCN1A* and *SCN1B*. Structure/function analysis in heterologous expression systems and in transgenic mice will be required in order to elucidate the precise kinetic and physiological consequences of these mutations.

The present study confirms the role that *SCN1A* plays in the etiology of GEFS+. The phenotypic variability in families with GEFS+ raises the possibility that the incidence of GEFS+ has been underestimated, since, in the absence of a complete family medical history, individuals with GEFS+ may be misclassified as manifesting one of the more common syndromes of IGE. *SCN1A* should also be considered as a candidate for other inherited epilepsy syndromes associated with FC, which were not tested in the present study. When functional assays for functional domains in the cytoplasmic loops of the sodium channels are developed, it will be of great interest to determine whether the variants described here influence channel function. The SNPs and the physical map of the sodium-channel gene cluster will be useful for testing the contributions that these genes make to other inherited disorders. Studies of allelic mutations of *SCN8A* in the mouse have demonstrated that mutations of a single sodium channel can generate a variety of neurological symptoms (e.g., ataxia, tremor, a dystonic movement disorder, and paralysis) (Meisler et al. 1997, and in press). We anticipate that mutations in the human sodium channels will be found to contribute to a broad spectrum of inherited disease.

Acknowledgments

We thank Birgit Rau for the DNA preparation and Dr. Margit Burmeister for helpful comments on the manuscript. This work was funded by research grants from the German Volkswagen-Stiftung (to A.H.), the Deutsche Forschungsgemeinschaft (grant Sa434/2-2 to T.S.), and the March of Dimes (to M.H.M.). A.E. acknowledges a fellowship from the American Epilepsy Society, with support from UCB Pharma, Inc. B.T.M. is a predoctoral fellow of the Genetics Training Program at the University of Michigan (grant T32 GM07544).

Electronic-Database Information

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

BLAST, http://www.ncbi.nlm.nih.gov/BLAST/ (for alignment of cDNA and genomic sequences)
GenBank Overview, http://www.ncbi.nlm.nih.gov/Genbank/

GenbankOverview.html (for 1204 residues in human *SCN2A* [accession number M94055], human *SCN3A* [AJ251507], *SCN4A* [accession number M81758], human *SCN5A* [accession number M77235], human *SCN8A* [accession number AB027567.1], human *SCN9A* [accession number X82835], human *SCN10A* [accession number AF117907.1], human *SCN11A* [accession number AF188679], *Fugu rubripes* [accession number D37977], *Electrophorus electricus* [accession number M22252], *Drosophila melanogaster* [accession number M32078], and *Polyorchis penicillatus* [accession number AF047380])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for GEFS+ [MIM 604236] and GEFS+ type 2 [MIM 604236 and MIM 604233])

References

Bulman DE (1997) Phenotype variation and newcomers in ion channel disorders. Hum Mol Genet 6:1679–1685

Commission on Classification and Terminology of the International League against Epilepsy (1989) Proposal for revised classification of epilepsies and epileptic syndromes. Epilepsia 30:389–399

Consensus Statement (1980) Febrile seizures: long term management of children with fever-associated seizures. Pediatrics 66:1009–1012

Escayg A, De Waard M, Lee DD, Bichet D, Wolf P, Mayer T, Johnston J, Baloh R, Sander T, Meisler MH (2000*a*) Coding and noncoding variation of the human calcium-channel β_4-subunit gene *CACNB4* in patients with idiopathic generalized epilepsy and episodic ataxia. Am J Hum Genet 66: 1531–1539

Escayg A, MacDonald BT, Meisler MH, Baulac S, Huberfeld G, An-Gourfinkel I, Brice A, LeGuern E, Moulard B, Chaigne D, Buresi C, Malafosse A (2000*b*) Mutations of *SCN1A*, encoding a neuronal sodium channel, in two families with GEFS+2. Nat Genet 24:343–345

Haug K, Hallmann K, Horvath S, Sander T, Kubisch C, Rau B, Dullinger J, Beyenburg S, Elger CE, Propping P, Heils A (2000) No evidence for association between the KCNQ3 gene and susceptibility to idiopathic generalized epilepsy. Epilepsy Res 42:57–62

Kearney JA, Plummer NW, Smith MR, Kapur J, Cummins TR, Waxman SG, Goldin AL, Meisler MH (2001) A gain-of-function mutation in the sodium channel gene *Scn2a* results in seizures and behavioral abnormalities. Neuroscience 102: 307–317

Kohrman DC, Smith MR, Goldin AL, Harris J, Meisler MH (1996) A missense mutation in the sodium channel Scn8a is responsible for cerebellar ataxia in the mouse mutant *jolting*. J Neurosci 16:5993–5999

McNamara JO (1999) Emerging insights into the genesis of epilepsy. Nature 399:A15–A22

Meisler MH, Kearney J, Escayg A, MacDonald BT, Sprunger LK. Sodium channels and neurological disease: insights from Scn8a mutations in the mouse. Neuroscientist (in press)

Meisler MH, Sprunger LK, Plummer NW, Escayg A, Jones JM (1997) Ion channel mutations in mouse models of inherited neurological disease. Ann Med 29:569–574

Peiffer A, Thompson J, Charlier C, Otterud B, Varvil T, Pappas C, Barnitz C, Gruenthal K, Kuhn R, Leppert M (1999) A locus for febrile seizures (FEB3) maps to chromosome 2q23-24. Ann Neurol 46:671–678

Plummer N, Meisler MH (1999) Evolution and diversity of mammalian sodium channel genes. Genomics 57:323–331

Ratcliffe CF, Qu Y, McCormick KA, Tibbs VC, Dixon JE, Scheuer T, Catterall WA (2000) A sodium channel signaling complex: modulation by associated receptor protein tyrosine phosphatase β. Nat Neurosci 3:437–444

Sampo B, Tricaud N, Leveque C, Seagar M, Couraud F, Dargent B (2000) Direct interaction between synaptotagmin and the intracellular loop I-II of neuronal voltage-sensitive sodium channels. Proc Natl Acad Sci USA 97:3666–3671

Sander T, Berlin W, Ostapowicz A, Samochowiec J, Janz D, Gscheidel N, Hoehe MR (2000) Variation of the genes encoding the human glutamate EAAT2, serotonin and dopamine transporters and susceptibility to idiopathic generalized epilepsy. Epilepsy Res 41:75–81

Scheffer IE, Berkovic SF (1997) Generalized epilepsy with febrile seizures plus: a genetic disorder with heterogeneous clinical phenotypes. Brain 120:479–490

Smith RD, Goldin AL (1997) Phosphorylation at a single site in the rat brain sodium channel is necessary and sufficient for current reduction by protein kinase A. J Neurosci 17: 6086–6093

Wallace RH, Wang DW, Singh R, Scheffer IE, George AL Jr, Phillips HA, Saar K, Reis A, Johnson EW, Sutherland GR, Berkovic SF, Mulley JC (1998) Febrile seizures and generalized epilepsy associated with a mutation in the Na+-channel beta 1 subunit gene SCN1B. Nat Genet 19:366–370