A Second Locus for Familial Generalized Epilepsy with Febrile Seizures Plus Maps to Chromosome 2q21-q33

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Summary

We report a clinical and genetic study of a family with a phenotype resembling generalized epilepsy with febrile seizures plus (GEFS+), described by Berkovic and colleagues. Patients express a very variable phenotype combining febrile seizures, generalized seizures often precipitated by fever at age >6 years, and partial seizures, with a variable degree of severity. Linkage analysis has excluded both the β1 subunit gene (*SCN1B*) of a voltage-gated sodium (Na$^+$) channel responsible for GEFS+ and the two loci, *FEB1* and *FEB2,* previously implicated in febrile seizures. A genomewide search, under the assumption of incomplete penetrance at 85% and a phenocopy rate of 5%, permitted identification of a new locus on chromosome 2q21-q33. The maximum pairwise LOD score was 3.00 at recombination fraction 0 for marker D2S2330. Haplotype reconstruction defined a large (22-cM) candidate interval flanked by markers D2S156 and D2S2314. Four genes coding for different isoforms of the α-subunit voltage-gated sodium channels (*SCN1A, SCN2A1, SCN2A2,* and *SCN3A*) located in this region are strong candidates for the disease gene.

Introduction

The estimated prevalence of febrile seizures (FSs) (MIM 121210) in children of age <5 years is 3%–5% (Verity et al. 1985). Twin and family studies have suggested that there is an important genetic contribution to the etiology of this disorder (Hauser et al. 1985; Tsuboi and Endo 1991). Families have been identified in which this trait segregates with a mode of inheritance compatible with

a monogenic model. Recently, two loci responsible for FSs, *FEB1* and *FEB2,* were mapped on chromosomes 8q (Wallace et al. 1996) and 19p (Johnson et al. 1998; Kugler et al. 1998), respectively. Among children who experience FSs, 2%–7% develop epilepsy later in life (Cendes et al. 1995; Maher and McLachlan 1995). A new familial syndrome named "generalized epilepsy with febrile seizures plus" (GEFS+) has recently been defined (Scheffer and Berkovic 1997; Singh et al. 1999). In pedigrees with GEFS+, patients present with febrile seizures that may persist at age >6 years, associated with afebrile generalized seizures (tonic-clonic seizures, absences, myoclonic seizures, or atonic seizures) and, sometimes, a mixture of these types of seizures, leading to a clinical profile of myoclonic-astatic epilepsy. The mode of inheritance is autosomal dominant with incomplete penetrance and a high rate of phenocopy. Recently, a locus was identified, by linkage analysis, on chromosome 19q13, and a mutation was found in the β1-subunit gene (*SCN1B*) of a voltage-gated sodium (Na$^+$) channel (Wallace et al. 1998).

We report a family in which affected individuals in three successive generations express variable phenotypes combining febrile seizures, generalized seizures often precipitated by fever at age 6 years, and partial seizures, with a variable degree of severity. This family presents clinical similarities with families with GEFS+ that have been described elsewhere (Scheffer and Berkovic 1997; Singh et al. 1999). A genomewide search on autosomes, using semiautomated fluorescent genotyping, permitted identification of a new GEFS+ locus on chromosome 2q21-q33.

Subjects and Methods

Subjects

A large nonconsanguineous French family with 13 affected members over 3 generations, as well as 1 affected spouse, was identified during a national campaign organized by the French Généthon center. A simplified pedigree of the family is presented in figure 1. Clinical evaluation was performed at the Center of Epileptology of
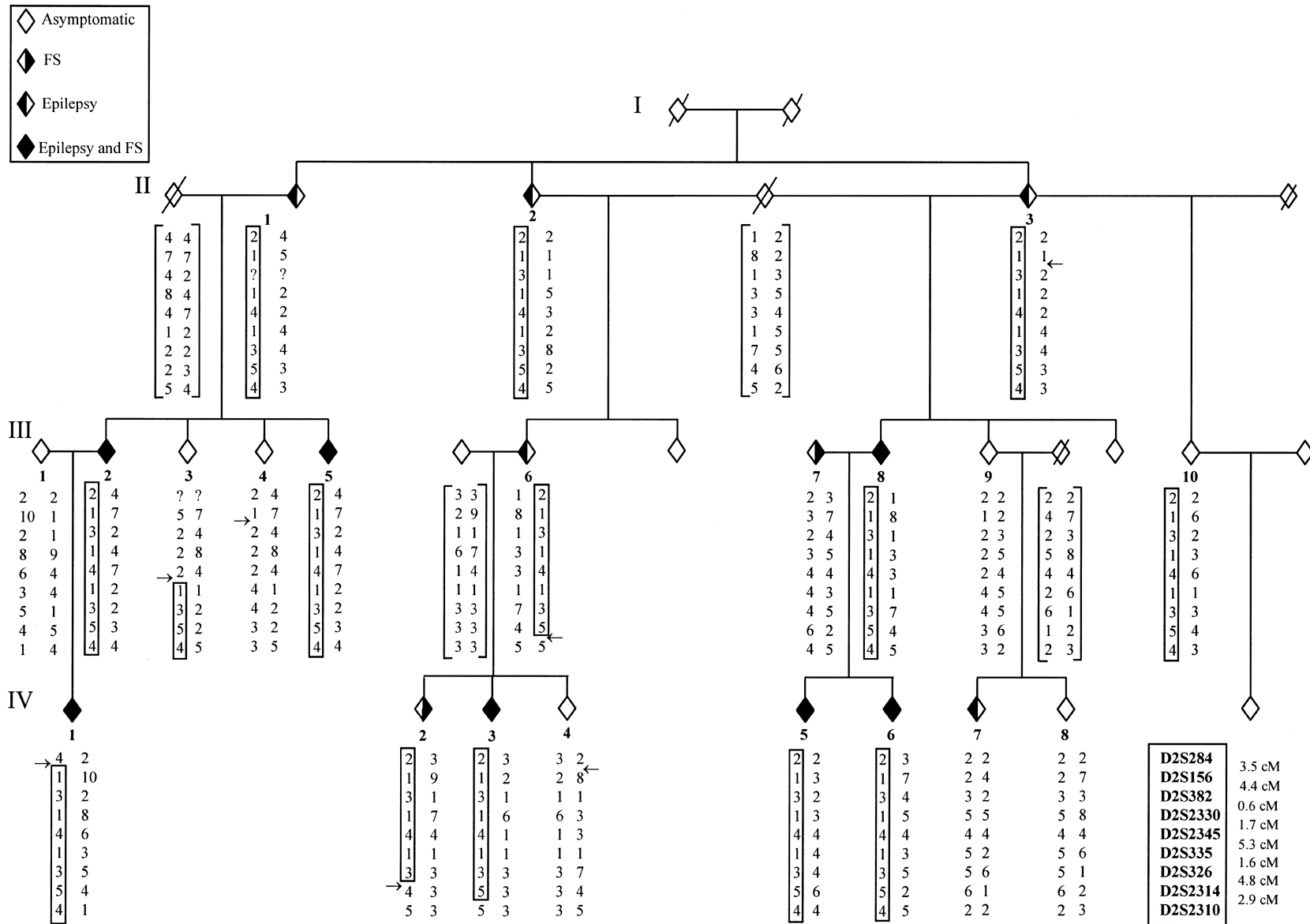
Figure 1 Partial pedigree of a French family with GEFS+, showing haplotype reconstruction for chromosome 2q markers. Deduced haplotypes are bracketed. Microsatellite markers are ordered, according to the Généthon genetic map, from centromere (top) to telomere (bottom). The haplotype segregating with the disease is boxed. Observed recombinations are indicated by arrows.

the Salpêtrière Hospital (Paris). All members of the family or their close relatives were contacted and underwent clinical assessment by means of a detailed questionnaire. Information was also obtained retrospectively from medical records. Electroencephalography (EEG) and neuroimaging were available for 7 and 6 of 13 patients, respectively. Informed consent was obtained from all participants or their legal representatives.

Genotyping

Blood samples from 21 subjects were obtained, and genomic DNA was extracted, by means of standard procedures. Family members were genotyped with the following microsatellite markers: *D8S553, D8S1840,* and *D8S530* and *D19S565, D19S591, D19S209, D19S216,* and *D19S177,* for the *FEB1* locus on chromosome 8q (Wallace et al. 1996) and the *FEB2* locus on chromosome 19p (Johnson et al. 1998), respectively, which are two loci responsible for familial FSs. The chromosome 19q locus involved in GEFS+ was tested with markers *D19S414, D19S868,* and *D19S425* (Wallace et al. 1998).

A genomewide search was performed with the ABI PRISM linkage-mapping set, version 2, from PE Biosystems. The set consists of 400 fluorescent microsatellite markers (including 20 markers for chromosome X that were not tested), selected from the Généthon human linkage map (Weissenbach 1993; Gyapay et al. 1994; Dib et al. 1996), that cover the entire human genome, with a resolution of ~10 cM (Schuster 1998). The markers were amplified by PCR under the following conditions: 50 ng of genomic DNA, 5 pmol of each primer, 2.5 mM of each dNTP, 1.5 μl of 10 × PCR buffer II (1.5 mM MgCl$_2$), and 0.6 U of Ampli*Taq* Gold DNA polymerase, in a final volume of 15 μl. Samples were incubated in a thermocycler for 12 min at 95°C, to activate the Ampli*Taq* Gold DNA polymerase; then for 15 s at 94°C, 15 s at 55°C, and 30 s at 72°C, for 10 cycles; and then for 15 s at 89°C, 15 s at 55°C, and 30 s at 72°C, for 25 cycles, followed by a final extension for 10 min at 72°C. After amplification, PCR products from each set were pooled with the GeneScan 400HD size standard and were loaded onto a 4% denaturing acrylamide gel, for electrophoresis with the ABI PRISM 377 DNA Sequencer (PE Biosystems). DNA from Centre d'Étude du Polymorphisme Human individual 1347-02 was used as an internal control. For fine mapping, five additional fluorescent markers from the chromosome 2q were selected from the Généthon linkage map. PCR amplification was performed as described above.

Linkage Analysis

Pairwise and multipoint LOD scores were calculated by the MLINK and LINKMAP programs of the FAST-

LINK package, version 3.0 (Schaffer et al. 1994), under the assumption of an autosomal dominant trait (male-to-male transmission excluded X-linked inheritance) with disease-allele frequency of .0001 and incomplete penetrance. Penetrance, calculated according to the method of Johnson et al. (1996), was estimated at 85%. Since the frequency of febrile seizures in the general population is ~3%–5%, we defined the phenocopy rate as 5%. Recombination fraction (θ) values were considered to be equal in males and females. Since simulation studies assuming these parameters indicated a maximum LOD score of 4.01 at θ = .00, with a five-allele marker (equal allele frequencies), a whole-genome scan, excluding chromosome X, was performed, under the assumption that the frequencies of the alleles observed in the family studied were equal. For markers with suggestive linkage, allele frequencies were calculated from a white population and were determined on the basis of The Genome Database. For multipoint analysis with markers from the candidate region, genetic distances from the Généthon linkage map were used: D2S142–8.6 cM–D2S382–0.6 cM–D2S2330–16.3 cM–D2S2310. Calculations were performed at the Lovelace Infobiogen Center (Villejuif, France). Haplotypes were reconstructed according to the principles of Thomson (1987). The order of markers was that of the consensus Centre d'Étude du Polymorphisme Human/Généthon chromosome 2 linkage map (table 1). The database of the Cooperative Human Linkage Center was searched for candidate genes within the linked interval.

Results

Clinical Data

Clinical data are summarized in table 2. The age at examination of affected individuals (11 females, including spouse III-7, and 3 males) was 12–77 years; 9 had histories of FS, with onset at age 4 mo–3 years, and 7

Table 1

Pairwise LOD Scores for 10 Markers on Chromosome 2

MARKER[a]	LOD SCORE AT θ =[b]						
	.00	.01	.05	.10	.20	.30	.40
D2S142	−2.14	−.61	.01	.23	<u>.33</u>	.28	.16
D2S284	−.45	−.27	.10	.30	<u>.42</u>	.36	.21
D2S156	<u>1.70</u>	1.69	1.62	1.49	1.18	.81	.42
D2S382	<u>1.98</u>	1.94	1.76	1.52	1.00	.50	.16
D2S2330	<u>3.00</u>	2.95	2.77	2.51	1.93	1.26	.56
D2S2345	<u>2.11</u>	2.08	1.94	1.74	1.26	.74	.29
D2S335	<u>.65</u>	.65	.65	.62	.47	.26	.09
D2S326	<u>2.17</u>	2.15	2.06	1.91	1.50	.99	.46
D2S2314	<u>.48</u>	.53	.66	.70	.60	.39	.19
D2S2310	−1.66	−.68	−.08	.12	<u>.18</u>	.11	.03

[a] Order of markers is from telomere (top) to centromere (bottom).
[b] Maximum LOD scores are underlined.

Table 2

Clinical Characteristics of Patients

SUBJECT (SEX, AGE [YEARS])	FS				EPILEPTIC SEIZURES				INTERICTAL EEG	BRAIN IMAGING (TECHNIQUE)[a]
	Status	Patient Age at		No.	Patient Age at		Pattern	No.		
		First Occurrence	Last Occurrence		First Occurrence	Last Occurrence				
II-1 (F, 69)	Unknown	...	...	...	60 years	60 years	GTCS	2	Not available	Not available
II-2 (F, 76)	Unknown	...	...	...	Childhood	76 years	GTCS	Numerous	Not available	Not available
II-3 (F, 77)	Unknown	...	...	...	25 years	30 years	GTCS	3	Not available	Not available
III-2 (F, 39)	Present	9 mo	...	Several	Childhood[b]	32 years	GTCS[b]	Numerous	Not available	Not available
III-5 (F, 32)	Present	4 mo	...	Numerous	Childhood[b]	32 years	GTCS,[b] versive seizures and left hemiclonic seizures	Numerous	Generalized spike-waves, right frontal focus	Normal (CT)
III-6 (F, 48)	Unknown	...	...	...	Childhood	46 years	GTCS	Several	Slow generalized spikes and waves, photosensitivity	Normal (CT)
III-7 (F, 41)	Present	3 years	3 years	1	...	...	...	...	...	...
III-8 (M, 45)	Present	2 years	Unknown	Several	Childhood	7 years	GTCS	Few	Not available	Not available
IV-1 (F, 19)	Present	1 year	3 years	Several	3 years	19 years	GTCS absences, absence status, right hemiclonic seizures	Numerous	Generalized spike-waves, 3-Hz generalized discharges	Slight cerebellar atrophy[c] (MRI)
IV-2 (F, 18)	Present	9 mo	4 years	Several	...	...	...	...	...	...
IV-3 (F, 23)	Present	7 mo	...	Numerous	7 mo?[b]	10 years[b]	GTCS[b]	Several	Normal	Not available
IV-5 (M, 18)	Present	1 year	...	Several	Childhood[b]	18 years	GTCS,[b] myoclonic seizures, atypical absences	Numerous	Slow-background, generalized spike-waves	Small right putaminal ischemic lesion (MRI)
IV-6 (F, 12)	Present	8 mo	...	Few	Childhood[b]	9 years	GTCS[b]	Numerous	3-Hz spike-waves	Normal (CT)
IV-7 (M, 24)	No	...	...	...	24 years	24 years	GTCS	4[d]	Normal	Normal (CT)

[a] CT = computed tomography; MRI = magnetic-resonance imaging.

[b] No symptom-free interval between FS and epileptic seizures, which were often precipitated by fever.

[c] Perhaps caused by chronic phenytoin treatment.

[d] One seizure precipitated by sleep deprivation and two seizures precipitated by abrupt cessation of antiepileptic drug.

of these 9 later developed epilepsy. The number of FS episodes experienced by each patient was highly variable. Two patients (III-5 and IV-5) had complex FS with prolonged or multiple attacks over 24-h periods. The occurrence of FS in the oldest patients (II-1, II-2, II-3, and III-6) could not be determined. Individual IV-7 never experienced FS.

In the 12 patients who developed afebrile seizures, the epilepsy phenotype was variable. The age at which the first epileptic seizure occurred could be determined for four individuals (II-1, II-3, IV-1, and IV-7) and was 3–60 years. For the other patients (II-2, III-2, III-5, III-6, III-8, IV-3, IV-5, and IV-6), age at onset could not be reliably recorded, because of either lack of precise information or lack of a seizure-free interval between FS and onset of epilepsy (with seizures often precipitated by hyperthermia) or because of an early association of FS and afebrile seizures. Generalized tonic-clonic seizures (GTCSs) occurred in all the epileptic patients. These seizures were associated with absences in two individuals (IV-1 and IV-5) and with myoclonic seizures (precipitated by carbamazepine) in patient IV-5. In four patients (III-5, III-6, IV-1, and IV-6), the results of interictal EEG were suggestive of idiopathic generalized epilepsy (normal background, generalized 3-Hz spike-waves, and photosensitivity for individual III-6). For individual IV-5, generalized spike-waves were associated with a slow background. In two individuals, some characteristics of partial seizures were associated with the generalized electroclinical pattern: right hemiclonic seizures in patient IV-1 and left hemiclonic and versive seizures with an interictal frontal focus in individual III-5. In both patients, no focal lesions or brain asymmetries were found on neuroimaging. Four patients were intellectually impaired (severely in the case of individual II-2 and moderately in the case of individuals III-5, IV-1, and IV-5), and one individual (IV-1) presented a pyramidal syndrome on the right side.

There was considerable intrafamilial variation in the severity of the epileptic disease, with respect to the frequency of seizures, clinical evolution, and response to antiepileptic drugs. The clinical evolution of seven patients was favorable. They had been seizure free during the 2 years preceding our study. Three of these seven patients were no longer taking antiepileptic drugs (individuals III-2, III-8, and IV-3), two were receiving monotherapy (individuals II-1 and II-3), and two were receiving antiepileptic-drug polytherapy (individuals III-6 and IV-6). In three patients (II-2, III-5, IV-1), a few seizures persisted during antiepileptic polytherapy. Patient IV-5 had pharmacoresistant epilepsy with numerous seizures persisting during antiepileptic polytherapy. At-risk individuals III-3, III-4, III-10, IV-4, and IV-8 were clinically normal at ages 44, 41, 56, 24, and 30 years, respectively, and were considered to be unaffected.

Linkage Analysis

The two loci previously reported for FS, *FEB1* on chromosome 8q (Wallace et al. 1996) and *FEB2* on chromosome 19p (Johnson et al. 1998), as well as the first locus identified for GEFS+, on chromosome 19q (Wallace et al. 1998), were first excluded by bipoint and multipoint analysis (data not shown). A scan of the entire genome, except for the X chromosome, which was excluded because of a male-to-male transmission, was conducted, with 380 markers, on the affected individuals and, when available, their parents. Pairwise LOD scores were calculated for all markers. Positive pairwise LOD scores were obtained at $\theta = .00$, for 35/380 markers. They were excluded by haplotype reconstruction and multipoint analysis, except for two adjacent markers on chromosome 2. A maximum pairwise LOD score of 2.79 at $\theta = .00$ was generated for marker D2S2330, and a positive LOD score of 1.46 at $\theta = .00$ was generated for marker D2S335. When all the available family members were included, the pairwise LOD scores reached the significant value of 3.00 at $\theta = .00$ for marker D2S2330 (table 1). The region underwent further analysis, with an increase of the density of genotyped markers. Positive LOD scores at $\theta = .00$ for five additional markers (D2S156, D2S382, D2S2345, D2S326, and D2S2314) in this region of chromosome 2 confirmed linkage (table 1). Multipoint analysis with markers D2S142, D2S382, D2S2330, and D2S2310 generated a LOD score of 3.03 between markers D2S382 and D2S2330 (fig. 2).

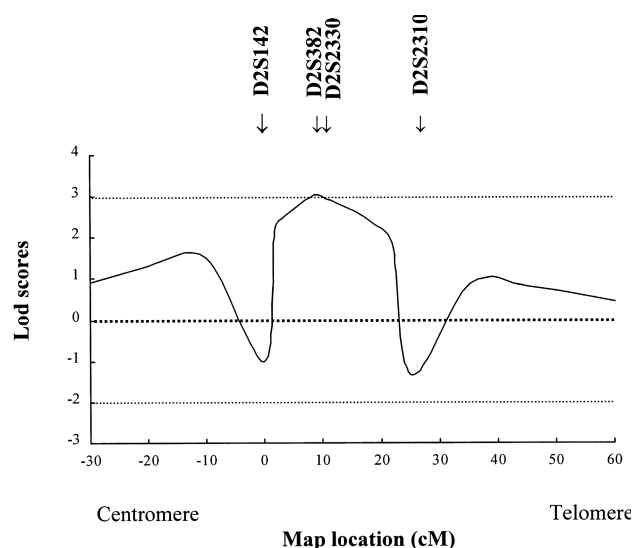Haplotype reconstruction showed that all patients

Figure 2 Multipoint analysis with chromosome 2q markers in the French family with GEFS+. The genetic distances are represented as on the Généthon chromosome 2 map. Horizontal dotted lines indicate the limit of exclusion (LOD score −2) and of significant linkage (LOD score 3).

except IV-7 and her unaffected mother, III-9, shared a common haplotype encompassing markers D2S156–D2S2314. The centromeric boundary of this interval was defined by a recombination between microsatellites D2S284 and D2S156 in patient IV-1, and the telomeric boundary was delimited by a recombination between markers D2S326 and D2S2314 in patient IV-2. This candidate interval corresponds to a 22-cM region that is localized on chromosome 2q21-q33, according to integrated maps of the Cooperative Human Linkage Center.

Discussion

The affected members of the family studied present a very heterogeneous epileptic phenotype, in which febrile convulsions were variably combined with several patterns of seizures. In some patients, the febrile seizures were peculiar either because they were associated, early in life, with afebrile generalized seizures, or because they persisted with afebrile seizures at age >6 years, as in the phenotype described and termed "febrile seizures plus" by Scheffer and Berkovic (1997). Within a family, FS+ may be variably associated with either generalized seizures (absences, myoclonic seizures, and atonic seizures) or clinical features resembling myoclonic-astatic epilepsy (Singh et al. 1999). The denomination "GEFS+" has been proposed for this new familial syndrome. The phenotype of the family that we studied resembles the GEFS+ syndrome but is singular because of the presence of partial seizures that are hemiclonic or versive in two affected subjects (III-5 and IV-1), suggesting involvement of the frontal lobe. Temporal-lobe seizures, reported in some GEFS+ families by Singh et al. (1999), were not observed. In addition to focal epileptic symptoms, both patients display several clinical and electrical features compatible with generalized idiopathic epilepsy (GTCSs without focal onset, absences, and/or generalized 3-Hz spike-waves). Moreover, intellectual impairment is present in some individuals of the family (i.e., individuals II-2, III-5, IV-1, and IV-5). Finally, for six patients (II-1, II-2, II-3, III-2, III-5, and III-6), seizures appeared or persisted later in life, compared with their occurrence in families previously described as having the GEFS+ syndrome.

After exclusion of known loci implicated in FS and GEFS+, a scan of all autosomes was undertaken, since a high rate of phenocopy could be expected in these syndromes. Pairwise analysis performed for 380 markers revealed positive LOD scores for 2 adjacent markers on chromosome 2, which reached a maximum value of 3.00 at $\theta = .00$ for microsatellite D2S2330. Haplotype reconstruction indicated that this second locus for GEFS+ is restricted to a 22-cM region between markers D2S156 and D2S2314. The presence of the haplotype associated with the disease in individual III-10, who is still asymptomatic at age 56 years, confirms that the penetrance is incomplete and/or age dependent. Because of the reduced penetrance, the recombination observed in 44 year-old asymptomatic individual III-3 does not definitively restrict the candidate region to the 10.2-cM interval between D2S156 and D2S2345.

Individual IV-7, who, in the linkage analysis, was considered to be affected, does not carry the haplotype segregating with the disease in the other patients, nor does his unaffected mother. He could therefore be considered to be a phenocopy. The clinical status of this 24-year-old patient is difficult to interpret: he has no history of febrile seizures, three of his four seizures were clearly precipitated by external events (see clinical data in table 2), and his EEG recording was strictly normal before initiation of antiepileptic-drug treatment. Genetic and clinical data support the hypothesis that he experienced provoked seizures rather than spontaneous epilepsy. Spouse III-7 was also considered to be a phenocopy in the linkage analysis, since she had only one FS and none of her relatives were affected, thereby excluding bilineal inheritance. This hypothesis is supported by the study by Rich et al. (1987), who showed that individuals with more than two FSs have a "nearly dominant" inheritance, whereas individuals with only a single FS, most of whom are sporadic cases, have multifactorial inheritance.

The identification of a new locus responsible for GEFS+ emphasizes the monogenic transmission and the genetic heterogeneity of this syndrome. A second family with GEFS+ linked to the same region has also been identified (B. Moulard, personal communication), suggesting that this locus might be a frequent cause of the disease. In addition, the localization, on chromosome 2, of a gene responsible for a form of idiopathic generalized epilepsy was reported in an abstract by Lopes-Cendes et al. (1995). It would be interesting to compare the phenotype and the chromosomal localization in the family that they studied with those in the family that we studied.

Several genes in the linked region of chromosome 2q21-q33 encode proteins implicated in the regulation of action potentials in the brain: SCN1A on 2q24, SCN2A1 on 2q23, SCN2A2 on 2q23-q24, and SCN3A on 2q24-q31. All four encode isoforms of a brain sodium-channel α-subunit (Han et al. 1991; Lu et al. 1992; Malo et al. 1994a, 1994b). Sodium channels isolated from vertebrate brain contain a large (230–270 kD) transmembrane α-subunit and one or two smaller (33–38 kD) β-subunits (Auld et al. 1988). Functional studies indicate that the α-subunit is sufficient to form a functional sodium channel (Hille 1992). These genes are attractive candidates for the GEFS+ syndrome in our family, since a mutation in the β1-subunit gene

(*SCNB1*) is known to cause GEFS+ in another family (Wallace et al. 1998). GEFS+ are most probably channelopathies, as are the vast majority of the familial idiopathic epilepsies for which the responsible genes are known (Steinlein et al. 1995; Charlier et al. 1998; Singh et al. 1998).

Acknowledgments

Electronic-Database Information

The accession number and URLs for data in this article are as follows:

Centre d'Étude du Polymorphisme Human, http://www.cephb.fr/ (for chromosome 2 linkage map and DNA from individual 1347-02)
Cooperative Human Linkage Center, The, http://lpg.nci.nih.gov/CHLC/ (for candidate genes and integrated maps)
Généthon, http://www.genethon.fr/ (for fluorescent microsatellite markers and chromosome 2 linkage map
Genome Database, The, http://www.gdb.org/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for FSs [MIM 121210])

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