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Mapping of a Gene Determining Familial Partial Epilepsy with Variable Foci to Chromosome 22q11-q12

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Summary

We identified two large French-Canadian families segregating a familial partial epilepsy syndrome with variable foci (FPEVF) characterized by mostly nocturnal seizures arising from frontal, temporal, and occasionally occipital epileptic foci. There is no evidence for structural brain damage or permanent neurological dysfunction. The syndrome is inherited as an autosomal dominant trait with incomplete penetrance. We mapped the disease locus to a 3.8-cM interval on chromosome 22q11-q12, between markers D22S1144 and D22S685. Using the most conservative diagnostic scheme, the maximum cumulative LOD score was 6.53 at recombination fraction (θ) 0 with D22S689. The LOD score in the larger family was 5.34 at $\theta = 0$ with the same marker. The two families share an identical linked haplotype for $\geqslant$10 cM, including the candidate interval, indicating a recent founder effect. A severe phenotype in one of the probands may be caused by homozygosity for the causative mutation, as suggested by extensive homozygosity for the linked haplotype and a bilineal family history of epilepsy. An Australian family with a similar phenotype was not found to link to chromosome 22, indicating genetic heterogeneity of FPEVF.

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Introduction

Idiopathic epilepsies may be partial or generalized and show specific, often age-related, clinical and electroencephalographic (EEG) features. Three types of idiopathic partial (localization-related) epilepsy were recognized by the Commission on Classification and Terminology of the International League Against Epilepsy (1989): benign childhood epilepsy with centrotemporal spikes, childhood epilepsy with occipital spikes, and primary reading epilepsy. More recently, an increasing number of idiopathic partial epilepsies has been described and subjected to intensive genetic investigation (Berkovic and Scheffer 1997*b*). These syndromes include: autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE [MIM 600513]) (Scheffer et al. 1994, 1995*a*), familial temporal lobe epilepsy (FTLE) (Berkovic et al. 1996; Andermann et al. 1997; Cendes et al. 1998), rolandic epilepsy with speech dyspraxia (MIM 601085) (Scheffer et al. 1995*b*), and familial partial epilepsy with variable foci (FPEVF) (Scheffer et al. 1998). Several idiopathic partial epilepsies show familial aggregation compatible with the effect of a major Mendelian gene (Berkovic and Scheffer 1997*a*), and in some, single-gene inheritance was confirmed by linkage studies and positional cloning. Mutations of the gene encoding the α-4 subunit of the neuronal nicotinic acetylcholine receptor on chromosome 20q were found to cause ADNFLE in an Australian (Phillips et al. 1995; Steinlein et al. 1995) and in a Norwegian family (Steinlein et al. 1997). A second locus for ADNFLE was more recently mapped to chromosome 15q, in the region where a cluster of genes encoding subunits of the neuronal nicotinic acetylcholine receptor is localized (Phillips et al. 1998). Because most families with ADNFLE do not show linkage either to chromosome 20 or to chromosome 15, at least one other, unmapped, locus must exist for this syndrome (Phillips et al. 1998). A gene determining a specific form of FTLE

called "partial epilepsy with auditory symptoms" (MIM 600512) has been mapped to chromosome 10q (Ottman et al. 1995). Preliminary results suggest linkage of FPEVF to chromosome 2 in an Australian family (Scheffer et al. 1998).

In an effort to contribute to the clinical and genetic definition of idiopathic partial epilepsy, we searched for families showing familial aggregation of idiopathic partial epilepsy. Here we report a linkage study of two large French-Canadian families sharing common ancestors that segregate a form of FPEVF as an autosomal dominant trait with reduced penetrance.

Patients and Methods

Ascertainment and Genealogy

The probands of families 22 and 14 were studied at the Montreal Neurological Hospital (MNH). Family trees were constructed, extending the pedigrees as many generations back as possible. The Australian FPEVF family was previously described by Scheffer et al. (1998).

Probands and Family Histories

We obtained seizure histories from all putatively affected and unaffected individuals, as well as from married-in spouses. We corroborated the histories by interviewing close relatives and reviewing medical records. Interviews were conducted at the MNH and during several field trips to the Quebec City region. To be classified as affected for linkage analysis, the records had to include at least a neurological and general examination, routine electroencephalogram (EEG), and a CT and/or magnetic resonance imaging (MRI) scan to exclude structural brain lesions. Four hospitalized patients, including the probands of families 22 and 14, also had a neuropsychological examination, sleep EEG, 24-hour video telemetry monitoring, volumetric MRI, and either functional MRI, magnetic resonance spectroscopy, or positron emission tomography for confirmation of epileptic foci.

The study protocol was approved by the institutional ethics committees, and informed consent was obtained from each individual or from a parent or guardian in the case of minors.

Family 22: proband and family history.—The proband is a 13-year-old boy from Saint-Raymond (Quebec, Canada), the second of two siblings. The parents are not known to be consanguineous. He was admitted for investigation of intractable epilepsy, which started at age 5 years. The seizures are mainly nocturnal and involve deep breathing, followed by pelvic movements, turning of the head to the right, and tonic flexion of the left arm and leg. Neurological and general examinations were normal. Video telemetry monitoring revealed bilateral frontal onset of seizures with right-sided predominance, followed by spread to the right temporal area. Interictal spikes and sharp waves were observed only in the right frontotemporal region. MRI, including volumetric MRI, revealed no structural lesions or asymmetries. A diagnosis of right frontal epilepsy without structural abnormalities was made.

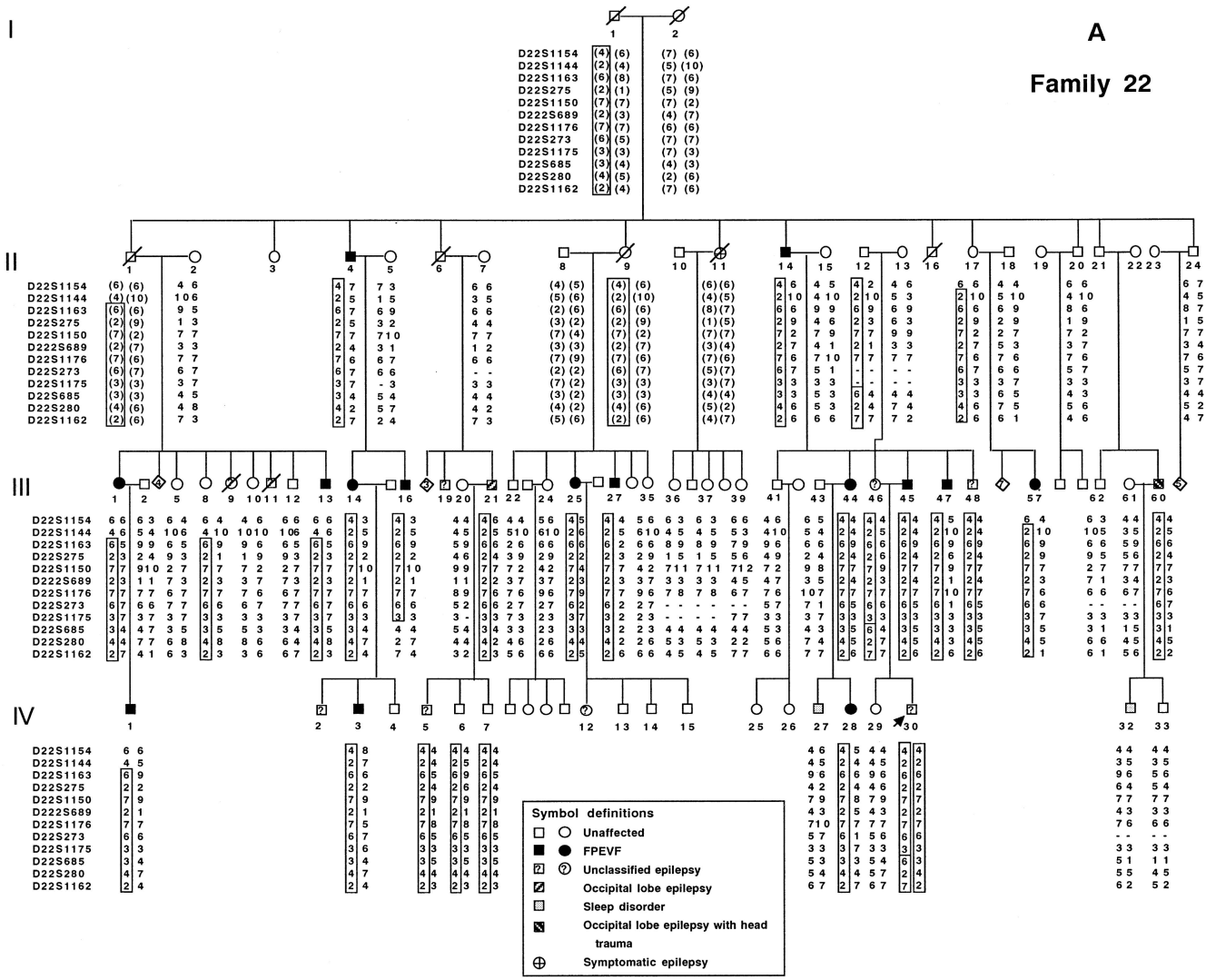
He had a family history of epilepsy. Among 119 family members (not including married-in spouses) in five generations, 25 individuals had seizures, including his father and paternal grandfather. Some relatives of his mother also had epilepsy, but no detailed investigations of this branch of the family have been carried out to date. Sleep disorders in childhood, three adults with positional vertigo, and three adults with paranoid schizophrenia were also identified in this family.

Family 14: proband and family history.—The proband is a 16-year-old male from Levis (Quebec, Canada), the youngest of three siblings whose parents are not known to be consanguineous. Seizures started at age 5 years with "absence" episodes. Over the years, he developed mainly nocturnal attacks occurring 5–10 times per month, characterized by tonic movements of the left upper extremity and, occasionally, of the left lower extremity and hemiface. Neurological and general examinations were normal. Video telemetry monitoring revealed an apparently generalized onset for clinical and electrographic seizures. An active interictal epileptic abnormality was recorded from the right frontotemporal convexity, often with simultaneous reflection in mesial temporal structures. MRI showed that the left hippocampus was smaller than the right. A diagnosis of right frontal epilepsy was made.

Family history was positive for epilepsy. Though most family members now live in Levis, some of their ancestors moved there from Saint-Raymond, where family 22 also originates. Of 79 family members (not including the married-in spouses), 11 in three generations had seizures, including the proband's father. Nonepileptic neurological or psychiatric disorders include: three family members with episodes of *déjà vu* and/or *jamais vu* but no overt seizures, eight family members who have classical migraine with visual auras, three children with sleep disorders, one child with febrile convulsions, and an adult with paranoid schizophrenia.

Definition of the Epileptic Syndrome

Clinical data were obtained for 34 individuals with a history of seizures, 24 from family 22 and 10 from family 14. All initial histories and EEGs were reported by one of four experienced epileptologists from Quebec City (R.D., G.P., S.V., and P.L.) and then reviewed and classified by three epileptologists from MNH (F.A., F.D., and S.F.B.). Consensus on affection status and locali-


A
Family 22
I
II
III
IV
D22S1154
D22S1144
D22S1163
D22S275
D22S1150
D22S689
D22S1176
D22S273
D22S1175
D22S685
D22S280
D22S1162
Symbol definitions
Unaffected
FPEVF
Unclassified epilepsy
Occipital lobe epilepsy
Sleep disorder
Occipital lobe epilepsy with head trauma
Symptomatic epilepsy

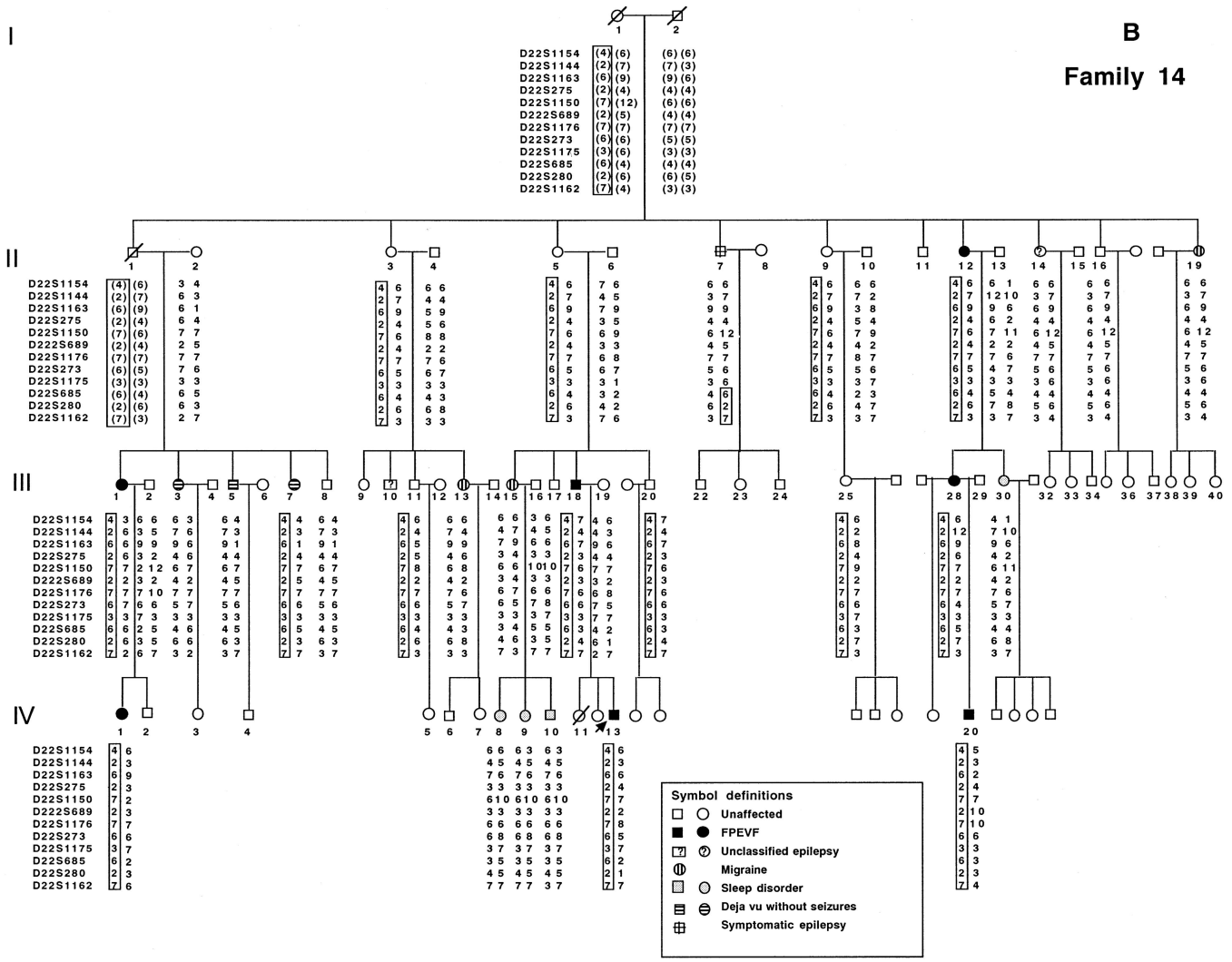


Figure 1 Pedigrees of two French-Canadian families segregating FPEVF. Only individuals indicated with blackened symbols were considered as affected in the analysis to establish linkage and then were used to identify the critical interval. Chromosome 22q11-q12 marker data are indicated below each typed individual. The disease-associated haplotype is boxed. In 22 individuals (panel *A*), two deduced paternal recombinations that define the centromeric boundary of the candidate region can be detected: one recombination occurred between D22S1144 and D22S1163 in II:1, the other between D22S1154 and D22S1144 in II:15. A paternal recombination between D22S685 and D22S280 occurred in III:16 and defines the telomeric boundary of the candidate interval. Note the homozygosity of the proband of family 22 (IV:30) for all but two distal markers. For distances between the markers, refer to figure 2. Inferred haplotypes are in parentheses.

zation of the epileptic focus was reached for each individual before the analysis of marker data. An effort was made to identify a set of core clinical characteristics that define the specific genetic syndrome, in order to exclude from primary mapping all cases whose phenotype or medical history suggests the possibility of a different etiology. Twenty-three individuals (16 from family 22 and seven from family 14) were found to share enough common clinical features to define a specific idiopathic partial epilepsy syndrome. DNA samples were available from all but one (from family 22). These individuals were classified as affected in the subsequent linkage analysis and are indicated with blackened symbols infigure 1. Their phenotypes include: (1) recurring partial seizures originating from different cortical areas; (2) absence of any detectable pathological lesion(s); (3) exclusively or predominantly nocturnal seizures; (4) variable age of onset, with two peaks around 5 and 25 years; (5) relatively inactive EEGs; (6) in most cases, a good response to therapy with antiepileptic medications (Xiong et al. 1998). The most peculiar characteristic of the syndrome is the variable localization of the epileptic focus in different family members, which prompted us to classify it as a form of FPEVF. Not all brain regions seem to be equally susceptible, because almost all patients have their epileptic focus in the frontal or temporal lobe, as indicated by the clinical characteristics of the seizures and, in four subjects (the two probands, one individual with frontal seizures, and one individual with temporal seizures), by video telemetry. Only one individual from family 22 (III:21 infig. 1*A*) has an occipital focus in the context of an otherwise compatible clinical picture. Because of this unusual presentation, he was classified as unknown for linkage analysis. Unknown status was also given to three subjects with symptomatic epilepsy (II:11 infig. 1*A*, who has post-stroke seizures; III:60 infig. 1*A*, who had occipital trauma before the onset of occipital epilepsy; II:7 infig. 1*B*, who has post-traumatic epilepsy), as well as to two subjects who have idiopathic or cryptogenic epilepsy with distinct clinical features (II:14 infig. 1*B*, who has temporal lobe epilepsy with onset at age 1 year and diurnal seizures; an individual from family 22 not shown infig. 1, who has typical benign childhood occipital epilepsy as described by Panayiotopoulos [1989]), and to two subjects who may have FPEVF by history, but whose records are insufficient (III:48 and IV:5 infig. 1*A*). The proband of family 22 (IV:30 infig. 1*A*) was also given unknown status, because his severe refractory epilepsy raised the suspicion of an undetected symptomatic origin and because both parents reported a family history of epilepsy, a possible confusing factor for linkage analysis. Clinical and EEG data were reviewed for seven individuals with episodic nonepileptic disorders such as classical migraine, isolated *déjà vu*, and parasomnias in childhood, and for 14 asymptomatic subjects (including married-in spouses). None had EEG abnormalities, and they were all considered unaffected. This diagnostic scheme (diagnostic scheme 1) was used for the purpose of gene mapping by linkage analysis.

In both families, the mode of inheritance of this idiopathic epileptic disorder appears to be autosomal dominant, as indicated by vertical and male-to-male transmission. Incomplete penetrance is suggested by the absence of a history of seizures in some obligate carrier individuals.

Genotyping and Molecular Methods

Genomic DNA was isolated by phenol-chloroform extraction (Gustafson et al. 1987). Manual screening of chromosomes 15–22 was performed with markers from the CHLC human screening set/Weber version 8, mostly tetra- and trinucleotide repeats. DNA fragments were amplified by PCR in a total volume of 12.5 μl with standard PCR buffer, a 0.2-μM concentration of each primer (one end-labeled with γ-^{32}P-ATP used at 0.5×10^6 cpm/reaction), a 0.125 mM concentration of each dNTP, 40 ng of DNA, and 0.5 U of *Taq* polymerase (Pharmacia). Thirty-three temperature cycles, consisting of 40 s either at 94°C, 55°C, and 72°C (tetra- and trinucleotide repeats) or at 94°C and 55°C (dinucleotide repeats), were used for PCR amplification. The amplified fragments were separated on 6%, 5%, or 4% polyacrylamide gels, depending on their size, in denaturing conditions. Radioactive PCR products were detected by autoradiography after electrophoresis. Part of the genomewide scan was performed using a screening set of fluorescently labeled markers (screening set-3, SS3), mostly tetra- and trinucleotide repeats, which were analyzed on an automatic ABI PRISM 377 sequencer (PE Biosystems) equipped with appropriate software.

The T→C polymorphism (position 836 of the mRNA sequence) in the ADORA2 gene (Genbank accession number U40771) was genotyped to resolve a contradictory localization of this gene in the GB4 and G3 radiation hybrid maps in GeneMap 98. Primers F (5′-TGG CAG TGA CGG AGA GCA GGT-3′) and R (5′-AGG AAG GGG CAA ACT CCA TGA-3′) from the second exon of the gene were used to amplify a DNA fragment containing the polymorphism. Genomic DNA was amplified by PCR in the conditions as described above for tetranucleotide markers, except that both primers were radioactively labeled and the annealing temperature was 60°C. Polymorphic variants were detected by single-strand DNA polymorphism (SSCP) analysis on 7% polyacrylamide gel, as described elsewhere (Zietkiewicz et al. 1992).

Linkage Analysis

We initially genotyped the families with markers from chromosomal regions harboring loci known to be linked to idiopathic partial epilepsy syndromes, particularly the suggested FPEVF locus on chromosome 2 (Scheffer et al. 1998), the ADNFLE loci on chromosomes 20 (Phillips et al. 1995) and 15 (Phillips et al. 1998), and the FTLE with auditory symptoms locus on chromosome 10 (Ottman et al. 1995). These loci were excluded by recombination and highly negative LOD scores (data not shown). We then proceeded to a whole-genome scan. Preliminary simulation analysis using the SLINK program (Weeks et al. 1990) indicated that family 22 had enough power to establish linkage when utilizing diagnostic scheme 1 and analysis parameters as described below. After linkage was established, markers from the linked region were tested on all available members of families 22 and 14 and on the Australian FPEVF family.

Linkage analysis was performed using the maximum-likelihood, parametric approach (via LOD score). Two-point LOD scores were calculated using the MLINK program (Lathrop et al. 1985), version 5.1. Diagnostic scheme 1 was initially used to define affected individuals for linkage analysis. After linkage was established, LOD scores were recalculated according to diagnostic schemes 2 and 3. Diagnostic scheme 2 considers as affected all individuals with idiopathic partial epilepsy, even if only by history, and regardless of severity and localization of the focus. Subjects with symptomatic epilepsy or with distinct clinical features are still excluded. This scheme adds to the affected group the subject with occipital seizures (III:21 infig. 1*A*), two individuals with history of partial epilepsy and no evidence of brain disease, but incomplete records (III:48 and IV:5 infig. 1*A*), and the proband of family 22 (IV:30 infig. 1*A*). In diagnostic scheme 3, every individual with epilepsy was considered affected. This scheme adds to the affected group the subjects with symptomatic epilepsy (II:11 and III:60 infig. 1*A* and II:7 infig. 1*B*) and the individual with early-onset, diurnal temporal lobe seizures (II:14 infig. 1*B*). A single autosomal dominant model with 50% penetrance was utilized in all cases, as suggested by inspection of the pedigrees. In both pedigrees, almost all individuals were typed, and the genotypes of a few deceased or unavailable persons could be unequivocally reconstructed by analyzing spouses and offspring (as shown infig. 1). Alleles were numbered as follows: the longest variant was assigned number 1, and all the shorter ones were numbered by adding the number of repeats by which they differed from allele 1. Alleles were assigned equal frequency in the LOD-score analysis. Haplotypes were constructed using the Cyrillic program (Cherwell Scientific) version 2, using the most parsimonious phase by tracing segregation of alleles in families.

Results

Linkage Analysis

Before significant evidence of linkage with D22S685 was found, 111 markers were tested (table 1). Next, we used 22 additional polymorphic markers on the long arm of chromosome 22, selected from the Généthon map (Dib et al. 1996) and from the Genetic Location Database to finely map the disease locus. Their physical order was obtained from the Sanger Centre physical map of chromosome 22. All markers are positioned in contig 22 in this map.Table 1 shows LOD scores obtained in families 22 and 14, with markers listed in cen→tel order according to the Sanger Centre map. With the most conservative diagnostic scheme (scheme 1), we obtained the highest cumulative LOD score of 6.53 for D22S689 at recombination fraction (θ) 0 (table 1). This marker generated a LOD score of 5.34 in family 22. Seven additional markers generated maximum LOD scores >3 in family 22: D22S275, D22S1150, D22S1176, and D22S273 at $\theta = 0$, D22S280 and D22S1162 at $\theta = .05$, D22S1144 at $\theta = .10$ (table 1). A candidate interval was defined by analysis of recombination events (fig. 1). Flanking markers are D22S1144 on the centromeric side, as shown by the recombinant haplotype of II:1 and his affected descendants III:1 and IV:1 (fig. 1*A*), and D22S685 on the telomeric side, as shown by the crossing-over that occurred in III:16 (fig. 1*A*). Affected individuals in family 22 and family 14, with the exception of those showing recombinations involving the more centromeric markers, share the same alleles in a region centromeric to D22S1175 and extending $\geqslant$10 cM, to D22S421 (figure 2). According to the Généthon map, in which D22S685 does not appear, the genetic distance between D22S1144 and D22S280, which is next to D22S685 in the Sanger Centre map, is 3.8 cM. The physical size of the D22S1144–D22S685 interval is ~4 Mb, according to the Sanger Centre map.

The adenosine receptor type 2 gene (ADORA2), a possible candidate gene whose physical location was uncertain because of contrasting results in the analysis of two radiation hybrid sets (GB4 and G3), was excluded from the candidate region. Several recombinants with the disease locus were identified by analysis of a polymorphism in the coding sequence of this gene, placing ADORA2 centromeric to the candidate interval.

LOD scores were recalculated according to diagnostic schemes 2 and 3 for the 12 linked markers shown intable 1 (seven in the critical interval). The application of diagnostic scheme 2 resulted in increased maximum LOD scores, with no changes in the recombination fractions

Table 1

LOD Scores between FPEVF and Chromosome 22 Markers in Two French-Canadian Families

Marker, Diagnostic Scheme, and Family	LOD Score at θ =[a]								
	0	.05	.10	.15	.20	.25	.30	Z_{max}	θ_{max}
D22S1154:									
1:									
22	−3.01	−.13	.43	.68	.77	.76	.67	.77	.20
14	1.78	1.73	1.62	1.48	1.30	1.09	.86	1.78	0
Overall	−1.24	1.60	2.05	2.15	2.07	1.85	1.53	2.15	.15
2:									
22	.13	1.99	2.18	2.14	1.99	1.75	1.43	2.18	.10
14	1.78	1.73	1.62	1.48	1.30	1.09	.86	1.78	0
Overall	1.90	3.72	3.80	3.62	3.29	2.84	2.29	3.80	.10
3:									
22	−.61	1.04	1.50	1.64	1.60	1.46	1.23	1.64	.15
14	−1.98	−.16	.31	.52	.61	.61	.55	.61	.20
Overall	−2.59	.88	1.81	2.16	2.21	2.07	1.78	2.21	.20
D22S1144:									
1:									
22	−.57	3.69	3.77	3.57	3.24	2.81	2.30	3.77	.10
14	1.78	1.73	1.62	1.48	1.30	1.09	.86	1.78	0
Overall	1.21	5.42	5.39	5.05	4.54	3.90	3.16	5.42	.05
2:									
22	2.07	5.32	5.11	4.72	4.21	3.61	2.94	5.32	.05
14	1.78	1.73	1.62	1.48	1.30	1.09	.86	1.78	0
Overall	3.85	7.05	6.74	6.19	5.51	4.70	3.80	7.05	.05
3:									
22	1.97	4.37	4.43	4.21	3.82	3.32	2.72	4.43	.10
14	−1.95	−.16	.31	.52	.61	.61	.54	.61	.20
Overall	.01	4.20	4.74	4.73	4.43	3.92	3.27	4.74	.10
D22S1163:									
1:									
22	2.90	2.66	2.40	2.12	1.82	1.50	1.16	2.90	0
14	1.08	.94	.78	.63	.48	.36	.25	1.08	0
Overall	3.98	3.59	3.18	2.74	2.30	1.85	1.41	3.98	0
2:									
22	3.88	3.52	3.15	2.76	2.35	1.93	1.50	3.88	0
14	1.08	.94	.78	.63	.48	.36	.25	1.08	0
Overall	4.97	4.46	3.93	3.39	2.84	2.29	1.75	4.97	0
3:									
22	1.98	2.57	2.47	2.25	1.96	1.64	1.28	2.57	.05
14	−.82	−.01	.13	.18	.19	.18	.16	.19	.20
Overall	1.16	2.56	2.60	2.43	2.15	1.82	1.44	2.60	.10
D22S275:									
1:									
22	3.32	3.90	3.77	3.48	3.10	2.65	2.14	3.90	0
14	1.78	1.73	1.62	1.48	1.30	1.09	.86	1.78	0
Overall	5.10	5.63	5.39	4.96	4.40	3.74	3.00	5.63	0
2:									
22	6.46	6.05	5.58	5.04	4.43	3.77	3.05	6.46	0
14	1.78	1.73	1.62	1.48	1.30	1.09	.86	1.78	0
Overall	8.24	7.78	7.20	6.51	5.73	4.86	3.91	8.24	0
3:									
22	4.56	5.10	4.90	4.52	4.04	3.48	2.83	5.10	.05
14	−1.98	−.16	.31	.52	.61	.61	.55	.61	.25
Overall	2.58	4.94	5.21	5.05	4.65	4.09	3.38	5.21	.10
D22S1150:									
1:									
22	3.18	2.96	2.71	2.43	2.13	1.80	1.45	3.18	0
14	1.18	1.17	1.12	1.02	.90	.74	.57	1.18	0
Overall	4.36	4.13	3.82	3.45	3.03	2.55	2.02	4.36	0

(*continued*)

Table 1 (Continued)

Marker, Diagnostic Scheme, and Family	LOD Score at θ =[a]								
	0	.05	.10	.15	.20	.25	.30	Z_{max}	θ_{max}
2:									
22	3.73	3.46	3.16	2.84	2.49	2.11	1.70	3.73	0
14	1.18	1.17	1.12	1.02	.90	.74	.57	1.18	0
Overall	4.91	4.63	4.28	3.86	3.38	2.85	2.27	4.91	0
3:									
22	3.73	3.46	3.16	2.84	2.49	2.11	1.70	3.73	0
14	−2.58	−.72	−.20	.07	.21	.26	.26	.26	.25
Overall	1.15	2.74	2.97	2.91	2.70	2.37	1.96	2.97	.10
D22S689:									
1:									
22	5.34	5.10	4.73	4.29	3.77	3.20	2.58	5.34	0
14	1.18	1.17	1.12	1.02	.90	.74	.57	1.18	0
Overall	6.53	6.28	5.85	5.31	4.67	3.95	3.15	6.53	0
2:									
22	7.42	6.86	6.25	5.59	4.89	4.13	3.32	7.42	0
14	1.18	1.17	1.12	1.02	.90	.74	.57	1.18	0
Overall	8.60	8.03	7.36	6.61	5.78	4.87	3.89	8.60	0
3:									
22	5.52	5.90	5.57	5.08	4.49	3.84	3.11	5.90	.05
14	−2.58	−.72	−.20	.07	.21	.26	.26	.26	.25
Overall	2.94	5.19	5.37	5.15	4.70	4.10	3.37	5.37	.10
D22S1176:									
1:									
22	3.15	2.93	2.69	2.41	2.12	1.79	1.44	3.15	0
14	.30	.28	.25	.23	.20	.17	.14	.30	0
Overall	3.45	3.21	2.94	2.64	2.32	1.97	1.59	3.45	0
2:									
22	3.15	3.02	2.83	2.59	2.31	1.99	1.63	3.15	0
14	.30	.28	.25	.23	.20	.17	.14	.30	0
Overall	3.45	3.29	3.08	2.82	2.51	2.16	1.77	3.45	0
3:									
22	3.15	3.02	2.83	2.59	2.31	1.99	1.63	3.15	0
14	.30	.28	.25	.23	.20	.17	.14	.30	0
Overall	3.45	3.29	3.08	2.82	2.51	2.16	1.77	3.45	0
D22S273:									
1:									
22	4.42	4.23	3.92	3.53	3.09	2.60	2.06	4.42	0
14	1.39	1.30	1.19	1.07	.93	.78	.61	1.39	0
Overall	5.80	5.53	5.11	4.60	4.02	3.38	2.67	5.80	0
2:									
22	6.43	5.91	5.34	4.75	4.11	3.44	2.73	6.43	0
14	1.39	1.30	1.19	1.07	.93	.78	.61	1.39	0
Overall	7.81	7.21	6.54	5.82	5.04	4.22	3.34	7.81	0
3:									
22	4.52	4.95	4.67	4.24	3.72	3.15	2.51	4.95	.05
14	1.39	1.30	1.19	1.07	.93	.78	.61	1.39	0
Overall	5.91	6.25	5.86	5.31	4.65	3.92	3.12	6.25	.05
D22S1175:									
1:									
22	.59	.52	.46	.39	.32	.26	.20	.59	0
14	.05	.08	.10	.10	.09	.08	.06	.10	.10
Overall	.64	.60	.55	.49	.41	.34	.26	.64	0
2:									
22	.84	.75	.66	.57	.48	.40	.31	.84	0
14	.05	.08	.10	.10	.09	.08	.06	.10	.10
Overall	.89	.83	.76	.67	.57	.47	.36	.89	0

(*continued*)

Table 1 (Continued)

Marker, Diagnostic Scheme, and Family	LOD Score at θ =[a]								
	0	.05	.10	.15	.20	.25	.30	Z_{max}	θ_{max}
3:									
22	.84	.75	.66	.56	.47	.38	.28	.84	0
14	−.30	−.29	−.27	−.23	−.19	−.14	−.09	−.09	.30
Overall	.54	.46	.39	.33	.28	.24	.19	.54	0
D22S685:									
1:									
22	.14	.93	1.04	1.03	.95	.83	.69	1.04	.10
14	1.48	1.45	1.37	1.25	1.10	.92	.71	1.48	0
Overall	1.62	2.38	2.41	2.28	2.05	1.75	1.40	2.41	.10
2:									
22	.51	1.32	1.42	1.38	1.27	1.10	.90	1.42	.10
14	1.48	1.45	1.37	1.25	1.10	.92	.71	1.48	0
Overall	1.99	2.77	2.79	2.63	2.36	2.02	1.61	2.79	.10
3:									
22	−1.34	.37	.74	.87	.88	.81	.68	.88	.20
14	−.13	.77	.94	.97	.91	.80	.64	.97	.15
Overall	−1.47	1.14	1.69	1.84	1.79	1.60	1.32	1.84	.15
D22S280:									
1:									
22	3.47	4.03	3.87	3.56	3.16	2.69	2.17	4.03	.05
14	1.78	1.73	1.62	1.48	1.30	1.09	.86	1.78	0
Overall	5.25	5.75	5.49	5.04	4.46	3.79	3.03	5.75	.05
2:									
22	3.97	4.54	4.39	4.06	3.64	3.12	2.54	4.54	.05
14	1.78	1.73	1.62	1.48	1.30	1.09	.86	1.78	0
Overall	5.75	6.27	6.01	5.54	4.94	4.22	3.40	6.27	.05
3:									
22	2.14	3.59	3.71	3.55	3.25	2.83	2.32	3.71	.10
14	.17	1.05	1.20	1.20	1.11	.97	.79	1.20	.10
Overall	2.31	4.64	4.91	4.75	4.36	3.80	3.11	4.91	.10
D22S1162:									
1:									
22	2.67	3.48	3.47	3.28	2.96	2.56	2.08	3.48	.05
14	1.18	1.15	1.07	.96	.82	.66	.49	1.18	0
Overall	3.85	4.63	4.55	4.24	3.79	3.22	2.58	4.63	.05
2:									
22	5.51	5.89	5.56	5.07	4.48	3.83	3.10	5.89	.05
14	1.18	1.15	1.07	.96	.82	.66	.49	1.18	0
Overall	6.69	7.04	6.63	6.03	5.31	4.49	3.59	7.04	.05
3:									
22	3.67	4.94	4.88	4.56	4.09	3.53	2.88	4.94	.05
14	−.43	.47	.65	.68	.64	.54	.42	.68	.15
Overall	3.24	5.41	5.53	5.24	4.73	4.07	3.30	5.53	.10

[a] Some entries do not sum to totals shown, because of rounding error.

at which they occurred. As only individuals from family 22 were added to the affected group when using this scheme, LOD scores changed only for this family. The highest LOD score for family 22 reached 7.42 and was obtained with D22S689 at $\theta = 0$. The corresponding cumulative LOD score for both families was 8.60. Compared to diagnostic scheme 1, family 22 generated a LOD score >3 with one more marker, D22S1163, at $\theta = 0$. When diagnostic scheme 3 was used, all maximum LOD scores for family 14 decreased and were obtained at higher recombination fractions. Maximum LOD scores also decreased for family 22 when compared with diagnostic scheme 2, but they were usually higher than with diagnostic scheme 1, despite a general increase in the recombination fractions at which they were obtained. This behavior is readily explained by looking at marker haplotypes. All subjects added to the affected group when applying diagnostic scheme 2 carry the disease-associated haplotype. The application of diagnostic scheme 3 added one more individual with the disease-associated haplotype to the affected group, III:60 from family 22 (fig. 1*A*), as well as three subjects without the

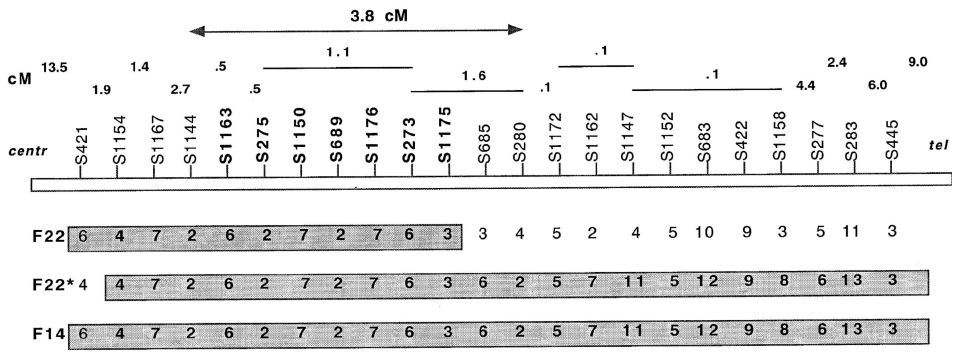


Figure 2 Disease haplotypes in families 22 and 14. The order of markers is based on the physical map of contig 22 in the Sanger Centre map, genetic distances (sex-averaged) are according to the Généthon map (Dib et al. 1996). F22 designates the disease haplotype in family 22, F22* the maternal haplotype of the proband of family 22. F14 designates the disease haplotype in family 14. Shared haplotype portions are gray. Markers within the candidate interval defined by recombination analysis are in bold. The 3.8-cM candidate region is marked by an arrow.

disease-associated haplotype, one from family 22 (II:11 infig. 1*A*), and two from family 14 (II:7 and II:14 infig. 1*B*).

Since multiple diagnostic schemes were used, a correction for multiple testing should be introduced when evaluating the significance of the results. Accordingly, LOD scores should be decreased by $\log_{10} 3$, this being the number of tested models. The resulting values for the highest LOD scores, obtained with diagnostic scheme 2, are 8.12 (cumulative) and 6.94 (family 22 only), both well above the cutoff of 3.3 proposed by Lander and Kruglyak (1995) for LOD-score analyses in humans. The corresponding genomewide *P* values are 1.2×10^{-7} and 1.7×10^{-5}, respectively (Lander and Kruglyak 1995).

Genotype-Phenotype Correlations

We genotyped all available members of families 22 and 14 with chromosome 22 markers to estimate the phenotypic spectrum of the partial epilepsy gene segregating in these families (fig. 1). In generation II (mean age 71 years, range 61–80), only 27% (3/11) of subjects carrying the linked marker haplotype had epilepsy compatible with the FPEVF syndrome. A history of epilepsy may be underreported in this generation, both for cultural reasons and because a few nocturnal partial seizures at a relatively young age might have been overlooked or been interpreted as sleep disturbance. In generation III, whose mean age is 43 years (range 32–56), 76% (16/21) of the subjects carrying the linked marker haplotype had epilepsy compatible with FPEVF. This generation carries fewer cultural constraints against reporting a history of epilepsy and seizures were experienced more recently. The individual with idiopathic occipital seizures in family 22 (III:21 infig. 1*A*), whose affection status was considered unknown for the mapping study because of the unusual location of the epileptic focus, was found to carry the linked haplotype. A second subject with occipital seizures from the same pedigree (III:60 infig. 1*A*), who had occipital head trauma, was also found to carry the disease-associated haplotype. Additional individuals excluded from the mapping study because of uncertainties in the diagnosis, but who had a history highly suggestive of FPEVF and were considered as affected in diagnostic scheme 2, were also found to carry the linked haplotype. Conversely, two individuals with possible symptomatic epilepsy, post-traumatic (II:7 infig. 1*B*) or post-stroke (II:11,fig. 1*A*), and a subject with a distinct syndrome of temporal lobe epilepsy (II:14 infig. 1*B*) were found not to carry the linked haplotype. Most subjects with nonepileptic episodic disorders or with isolated episodes of *déjà vu* also do not carry the linked haplotype.

The proband of family 22, who was excluded from the mapping study because of the severity and refractoriness to treatment of his epilepsy and because of a history of epilepsy in both parents' families, was found to be homozygous for all markers contained within the 7.8-cM interval between D22S1154 and D22S1175, which includes the entire critical region centromeric to D22S685. Considering the marker allele frequencies, this event is expected to occur by chance with a probability of less than 10^{-6}. Furthermore, his maternal haplotype matches the haplotype segregating with the disease in family 14 for $\geq$21 cM, from D22S1154 to D22S445, the most telomeric marker we tested (fig. 2), suggesting a close relationship between family 14 and maternal relatives of the proband of family 22, also from the town of Saint-Raymond. We propose that this individual may be homozygous for the causative mutation, resulting in a more severe phenotype. Several affected individuals are homozygous for two or three contiguous markers in the candidate interval. Such limited homozygosity very

likely occurred by chance, as none of these individuals has a severe phenotype or evidence of bilineal inheritance of epilepsy.

Discussion

We identified a specific epilepsy syndrome which appears to be determined by a single major gene and is characterized by predominantly nocturnal partial seizures originating from variable brain areas in the absence of detectable structural abnormalities. We called the syndrome familial partial epilepsy with variable foci (FPEVF), the term introduced by Scheffer et al. (1998) to classify an Australian family with similar phenotype. Rigorous and critical analysis of the clinical data was essential to achieve this result. Epilepsy is common, so the simple finding of multiple cases in a pedigree does not necessarily indicate that a specific genetic syndrome is segregating in the family. Furthermore, some affected individuals may have epilepsy of a different kind. The identification of a set of clinical features that characterize the syndrome, despite the variability of clinical presentations, was therefore a necessary prerequisite for the linkage study.

We chose the maximum-likelihood parametric method for linkage analysis—the most powerful method, for Mendelian disorders—for several reasons. First, inspection of the pedigrees strongly suggested that a single major autosomal dominant mutation with reduced penetrance was responsible for the epilepsy phenotype. Second, single-gene inheritance had previously been demonstrated for other idiopathic partial epilepsies. Third, the LOD-score method can be the most powerful as long as an appropriate model for the locus being tested is utilized, even if the "real" model involves several genes (Greenberg et al. 1998).

As for marker loci, the assumption of equal allele frequencies facilitated the calculations and is highly unlikely to have led to false positive results, because there were very few untyped individuals, and because their haplotypes could always be unequivocally inferred. In addition, results were highly consistent for all markers in the linked region.

Our linkage study provides strong evidence that a single major gene on chromosome 22 causes FPEVF in two French-Canadian pedigrees (14 and 22). Only after linkage was firmly established, by use of a conservative diagnostic scheme, did we use two more diagnostic schemes to define affected individuals for LOD-score analysis. Assuming that the model that generates the highest LOD score is the best approximation to the "real" characteristics of the trait under study, diagnostic scheme 2 seems to provide the best definition of the phenotype among those tested. Accordingly, FPEVF is characterized by nocturnal frontal, temporal, or occipital epilepsy of variable severity that is most often—but not always—responsive to treatment. Since the approach of maximizing the maximum LOD score with respect to the clinical parameters (Clarget-Darpoux et al. 1986; Greenberg 1990; Hodge and Elston 1994) carries an increased risk of false positive results, significance levels should be reevaluated after adjusting for the multiple models that were tested (Clerget-Darpoux et al. 1986). Thanks to the size of the families we studied, our results remain highly significant even after this correction.

The linked haplotype in the two families is the same across a region of $\geqslant$10 cM, pointing to a recent founder effect, probably coinciding with the establishment in the first half of the 19th century of the town of Saint-Raymond, from which both pedigrees originate. Interestingly, one individual, the proband of family 22, is homozygous for the linked haplotype, and epilepsy was present in relatives of both parents. This individual is highly likely to be homozygous for the causative mutation and is severely affected with intractable epilepsy.

Obviously , successful mapping of the major gene determining FPEVF in these families does not exclude the possibility that other genes influence the phenotype. This is, in fact, quite likely, when the observed reduced penetrance and variability of the phenotype are considered. It should come as no surprise that this idiopathic partial epilepsy syndrome—like many disorders caused by a single major gene with Mendelian inheritance—has a complex, multifactorial etiology that determines the specific phenotype of each gene carrier, in which modifier genes, acquired factors, and chance may all play a role.

The Australian FPEVF family described in Scheffer et al. (1998), which previously provided suggestive linkage for chromosome 2, was also tested for linkage to chromosome 22. This family was not found to be linked to chromosome 22q11-q12. Clinical findings that could differentiate it from our families 22 and 14 include: (1) a wider range of ages at onset, varying from 10 mo to 44 years and possibly to 55 years in one deceased individual; (2) predominantly diurnal seizures; (3) more interictal EEG activity even in individuals with frontal seizures. Overall, the genetic background of FPEVF is distinct from that of other idiopathic partial epilepsies: FPEVF is genetically, and probably clinically, heterogeneous.

The candidate interval on chromosome 22 covers a genetic distance of <4 cM between D22S685 and D22S1144 and has a physical size of ~4 Mb. The region is entirely within the Sanger Centre contig 22, and finished or unfinished sequence is available for most of it. Known genes mapping in this interval include those encoding (in cen→tel order): the X-box binding protein 1 (XBP1); the growth-arrest protein GAR22; the ras-related protein RRP22; an oncogene involved in Ewing sarcoma (EWS); β-adaptin (ADTBL); the neurofilament

heavy polypeptide (NEFH); the NF2 protein, merlin; the leukemia inhibitory factor (LIF); oncostatin M (ONCM); transcobalamin II (TCN2); smoothelin (SMTN); the brain-specific η isoform of the 14.3.3 protein, also known as tyrosine/ tryptophan mono-oxygenase activating protein (YWMAP); the sodium-glucose transporter 1 (SGLT1); the immunoglobulin λ chain (IGL); and the synaptic vesicle protein synapsin III.

We excluded the adenosine-receptor type 2 gene (ADORA2) from the candidate region because of multiple recombinations, detected by analysis of a coding-sequence polymorphism. The genes encoding synapsin III and the brain-specific η isoform of the 14.3.3 protein (YWMAP) are appealing candidates for FPEVF. After synapsins I and II, synapsin III is the most recently identified member of a gene family encoding proteins associated with synaptic vesicles that regulate neurotransmitter release (Kao et al. 1998). Knockout mice for synapsin I and synapsin II, as well as mice with the double knockout, are viable and fertile and have no structural brain abnormality but develop epilepsy (Rosahl et al. 1995). The severity of epilepsy in these knockout mice is proportional to the number of disrupted synapsin genes (Rosahl et al. 1995), suggesting parallelism with the observation of a severe phenotype in the proband of family 22, possibly homozygous for the mutation. The synapsin III gene is localized at the telomeric end of the candidate interval, with the flanking markers D22S280 and D22S685 lying in a very large intron between exons 5 and 6, according to the Sanger Centre map. Despite this peripheral location, it is not excluded because of the possibilities of intragenic recombination and of a recombination hot spot in this large intron.

The YWMAP gene is entirely within the candidate region, between D22S273 and D22S1175. It encodes a brain-specific protein with roles in the control of intracellular signaling and neurotransmitter release. Alternatively, a still-unidentified gene may be responsible. A large number of expressed sequence tags (ESTs) and of putative exons have been localized in the region, and their analysis will certainly speed the process of new gene identification.

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Electronic-Database Information

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

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim

GeneMap 99, http://www.ncbi.nlm.nih.gov/genemap99/

Généthon map, ftp://ftp.genethon.fr/pub/Gmap/Nature-1995

Genetic Location Database, ftp://cedar.genetics.soton.ac.uk/public_html/index.html

Sanger Centre map of chromosome 22, http://www.sanger.ac.uk/HGP/Chr22/

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