Genetic epidemiological approaches in complex neurological disorders

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CHAPTER 8

Familial partial epilepsy with variable foci in a Dutch family: clinical characteristics and confirmation of linkage to chromosome 22q

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Abstract

Purpose: Three forms of idiopathic partial epilepsy with autosomal dominant inheritance have been described: (1) autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE); (2) autosomal dominant lateral temporal epilepsy (ADLTE) or partial epilepsy with auditory features (ADPEAF); and (3) familial partial epilepsy with variable foci (FPEVF). Here, we describe linkage analysis in a Dutch four-generation family with epilepsy fulfilling criteria of both ADNFLE and FPEVF. *Methods:* Clinical characteristics and results of EEG, CT and MRI were evaluated in a family with autosomal dominantly inherited partial epilepsy with apparent incomplete penetrance. Linkage analysis was performed with markers of the ADNFLE (1p21, 15q24, 20q13.3) and FPEVF (2q36, 22q11-q12) loci. *Results:* Epilepsy was diagnosed in ten relatives. Age at onset ranged from three months to 24 years. Seizures were mostly tonic, tonic-clonic, or hyperkinetic with a wide variety in symptoms and severity. Most interictal EEGs showed no abnormalities but some showed frontal, central, and/or temporal spikes and spike-wave complexes. From two patients, an ictal EEG was available, showing frontotemporal abnormalities in one and frontal and central abnormalities in the other. Linkage analysis with the known loci for ADNFLE and FPEVF revealed linkage to chromosome 22q11-q12 in this family. *Conclusions:* The clinical characteristics of this family fulfilled criteria of both ADNFLE and FPEVF. The frequent occurrence of seizures during daytime and the observation of interictal EEG abnormalities originating from different cortical areas were more in agreement with FPEVF. The observed linkage to chromosome 22q11-q12 supported the diagnosis of FPEVF and confirmed that this locus is responsible for this syndrome.

Keywords

FPEVF, ADNFLE, clinical characteristics, genetics, chromosome 22

Introduction

Three forms of idiopathic partial epilepsy syndromes with autosomal dominant inheritance have been described: (1) autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE)[1,2]; (2) autosomal dominant lateral temporal epilepsy (ADLTE) or partial epilepsy with auditory features (ADPEAF)[3,4]; and (3) familial partial epilepsy with variable foci (FPEVF)[5,6] .

ADNFLE is characterized by clusters of brief tonic and hyperkinetic motor seizures occurring mostly during sleep[1,2]. Onset is usually in childhood and seizures often persist throughout adult life with considerable intra-familial variation in severity. The predominant finding of the ictal electroencephalogram (EEG) is bilaterally sharp wave activity localized in the anterior quadrants, whereas interictal EEGs usually do not show diagnostic epileptiform abnormalities. In a number of families and in one isolated patient, three different mutations in the gene encoding the α4-subunit of a neuronal nicotinic acetylcholine receptor (CHRNA4) on chromosome 20q13.3 have been described[7-13]. All these mutations are situated in the second transmembrane region[8,9,11]. Considerable locus heterogeneity was documented for ADNFLE. Two different mutations in the gene encoding the β2-subunit of the neuronal nicotinic acetylcholine receptor (CHRNB2) on chromosome 1p21 were found responsible for the epilepsy in two families with ADNFLE[14-16]. In 1998, another locus was found on chromosome 15q24 but the responsible gene has not yet been identified[17].

ADLTE or ADPEAF is characterized by simple partial seizures with auditory symptoms and secondary generalization[3,18-20]. Sensory and psychic symptoms may also occur. Age at onset is usually in the first two decades of life. In one family, the symptoms were accompanied by aphasia[21]. Interictal EEGs sometimes show temporo-occipital sharp wave activity. Because the seizure semiology strongly suggests a seizure origin in the lateral temporal lobe, the

syndrome has been described as 'autosomal dominant lateral temporal epilepsy' (ADLTE)[4]. The syndrome was linked to chromosome 10q22-q24 [3,4,19-21]. Recently, 11 different mutations in the leucine-rich glioma-inactivated 1 gene (LGI1) on chromosome 10q24 have been found responsible for this syndrome in 11 families, including the one with aphasic seizures[22-27]. The loss of both copies of this gene promotes glial tumor progression, leading to the assumption that this gene might function as a tumor-suppressor gene[28]. The role of LGI1 in the pathogenesis of epilepsy is still unknown. It is the first gene not apparently encoding an ion channel or neurotransmitter receptor that has been identified for a human idiopathic epilepsy syndrome.

Seizures in FPEVF have a wide range of age at onset and are often heterogeneous within families, both clinically and neurophysiological[5,6]. They can be nocturnal or diurnal and may be simple or complex partial, originating from temporal, frontal, occipital or centroparietal areas, with sometimes secondary generalization. Until now, one Australian family and two French-Canadian families with FPEVF have been described[5,6]. In the French-Canadian families seizures were predominantly nocturnal and interictal EEGs were often normal, while most affected relatives of the Australian family had diurnal seizures and abnormalities in the interictal EEG. The frequent occurrence of daytime seizures and the observation of interictal EEG abnormalities originating from different cortical areas distinguish FPEVF from ADNFLE. FPEVF is autosomal dominantly inherited with incomplete penetrance. Suggestive linkage of the syndrome to chromosome 2q36 was observed in the Australian family by Scheffer et al.[5] The French-Canadian families with FPEVF were linked to chromosome 22q11-q12 [6].

We describe a Dutch four-generation family with autosomal dominant partial epilepsy, fulfilling criteria of both ADNFLE and FPEVF. We tested linkage to the known ADNFLE loci at chromosome 1p21 (CHRNB2), 15q24 and

20q13.3 (CHRNA4) and the known FPEVF loci at chromosome 2q36 and 22q11-q12.

Patients and Methods

Patients

This family is included in a nationwide study of the genetics of idiopathic epilepsies in the Netherlands. After obtaining written informed consent, clinical characteristics of the seizures of all participating affected members and, if performed, results of EEG, computed tomography (CT) and magnetic resonance imaging (MRI) were obtained from the treating physician. Furthermore, all participating relatives and married-in spouses (n = 42) were personally interviewed by PC and OB about their medical history and the medical history of their children. Based on the clinical characteristics of the seizures and the results of EEG, CT, and MRI, seizures were classified according to the criteria of the International League Against Epilepsy[29]. This study has been approved by the medical ethical committee of the Leiden University Medical Center.

Genotyping

Venous blood samples were taken from affected relatives and some of the healthy relatives and spouses. Genomic DNA was extracted from peripheral lymphocytes using standard methods[30]. The loci of ADNFLE and FPEVF were tested by polymerase chain reaction (PCR) with the following microsatellite markers: D1S498, D1S305 and D1S2635 for the chromosome 1p21 region; markers D15S211, D15S1041, and D15S979 for the chromosome 15q24 region; markers D20S100, D20S443 and D20S171 for the chromosome 20q13.3 region; markers D2S130, D2S133, and D2S2228 for the chromosome 2q36 region; and markers D22S310, D22S1167, D22S1144, D22S1163, D22S275, D22S1176, D22S273, D22S280, D22S1686, and D22S1162 for the chromosome 22q11-q12 region. Oligonucleotide sequences were available through the Human Genome Database (GDB). PCRs for all markers were

performed using the same protocol. The reaction was performed in a 15-µl reaction volume, containing 7.5 pmol of each primer, 1x superTaq PCR Buffer I (Enzyme Technologies Ltd, UK), 1.3 M betaine (ICN Biomedical Inc, Ohio, USA), 3.00 mM of dNTPs, 0.25 U Silverstar (Eurogentech, Liège, BE) and 45 ng of genomic DNA. This mixture was subjected to ten cycles of 30 seconds at 94°C, 30 seconds at 55°C, and 40 seconds at 72°C, followed by 25 cycles of 30 seconds at 89°C, 30 seconds at 55°C, and 40 seconds at 72°C. The PCR was preceded by an initial denaturation step of ten minutes at 94°C and was ended with an extension step of ten minutes at 72°C. PCR products for each template were pooled and run in an Applied Biosystems (ABI) 377 or 3700 automated DNA sequencer. Allele sizes were determined on the basis of an internal standard size marker (Genescan 400 HD [rox] size standard), using GeneScan 3.5 and Genotyper 3.6 ABI software. Genotypes were determined by two individuals, and checked for Mendelian segregation using UNKNOWN version 5.03.

Linkage analysis

Two- and multipoint LOD score analysis was performed using the Linkage program, version 5.1 [31]. In the linkage analysis, only definitely affected relatives were considered as affected. Diagnosis in all other relatives was considered unknown. Based on the model of Xiong et al., the mode of inheritance was assumed to be autosomal dominant with 50% penetrance[6]. Furthermore, we used a phenocopy rate of 0.01 and a gene frequency of 0.001. Allele frequencies for each individual marker were calculated with ILINK. Multipoint analysis was performed with inter-marker distances according to the database of the Marshfield Center for Medical Genetics for the markers in all five regions (www.marshfieldclinic.org/research/ genetics).

Results

Case history

The proband of this family (IV:9), a 19-year-old male, experienced his first seizure at the age of three months. After breastfeeding, he became apneic and cyanotic for two minutes with generalized hypertonia and staring. The following months, the same occurred several times. The interictal EEG showed right temporal epileptic discharges. The patient received valproic acid and became seizure free.

At the age of three years, he developed short lasting (30-60 seconds) complex partial seizures with tonic-clonic movements of the left arm and leg, accompanied by deviation of the eyes and unconsciousness occurring several times daily, predominantly late in the evening and during the night. Valproic acid was restarted but seizures did not remit. Furthermore, the patient displayed autistic-like behavior with aggressiveness. An initial interictal EEG at that time showed no abnormalities but in a second, long-lasting, EEG intermittently occurring sharp waves were observed in the right centroparietotemporal region without clinical manifestations. Medication was switched to carbamazepine but secondary generalized nocturnal seizures continued. At the end of the seizures he sighed and continued sleeping. In addition, diurnal seizures occurred with staring, unresponsiveness, and motor automatisms for 30 seconds. Medication was changed to phenytoin but without any improvement. The EEG showed right temporal and occipital sharp waves and (poly) spike-wave complexes. Brain MRI was normal.

At the age of six years, the seizure pattern changed into clusters of approximately 40 short seizures (20-30 seconds) in the morning with flushing and distortion of the left corner of his mouth, during which he remained conscious. He used phenobarbital and valproic acid at that time. The ictal EEG showed short series of right frontotemporal 7-8 Hz paroxysmal activity. After increasing the valproic acid dosage, he became seizure free. One year later, the

phenobarbital was stopped and the dose of the valproic acid decreased. The EEG showed no abnormalities at that time.

At the age of 15 years, all medication was stopped. Six months later, seizures re-occurred but at a much lower frequency. At the age of 17 years, seizure frequency increased again up to 2-3 seizures per night. The EEG showed a slow background rhythm with right temporal sharp waves and spikes, after which the dose of the valproic acid was increased. The behavioral problems became worse. A second brain MRI showed no abnormalities.

At the time of last evaluation (19 years), he experienced seizures almost every night during which he often fell out of bed. He also had tonic-clonic seizures during daytime. He was treated with valproic acid and lamotrigine.

Description of the family

In 12 persons of this family epilepsy had been diagnosed; two of them were deceased (figure 1). The family showed autosomal dominant inheritance with incomplete penetrance. There were six obligate carriers (II:3, II:6, III:11, III:17, III:21, and III:27). All patients had normal intelligence and no known history of any condition that could have caused seizures. Computed tomography was performed in five patients and did not show any abnormalities. Magnetic resonance imaging was performed in four patients and showed infratentorial and occipital atrophy in one patient (III:14), which, however, was not associated with clinical symptoms. Three patients had psychiatric problems such as autistic behavior in two (IV:7 and IV:9) and an obsessive-compulsive disorder in one (II:2).

Age at onset of seizures ranged from three months to 24 years (median 7.3 years, table 1). Eight patients had nocturnal seizures with a wide variety of symptoms and severity, and nine patients suffered from diurnal seizures, in one of them occurring shortly after awakening.

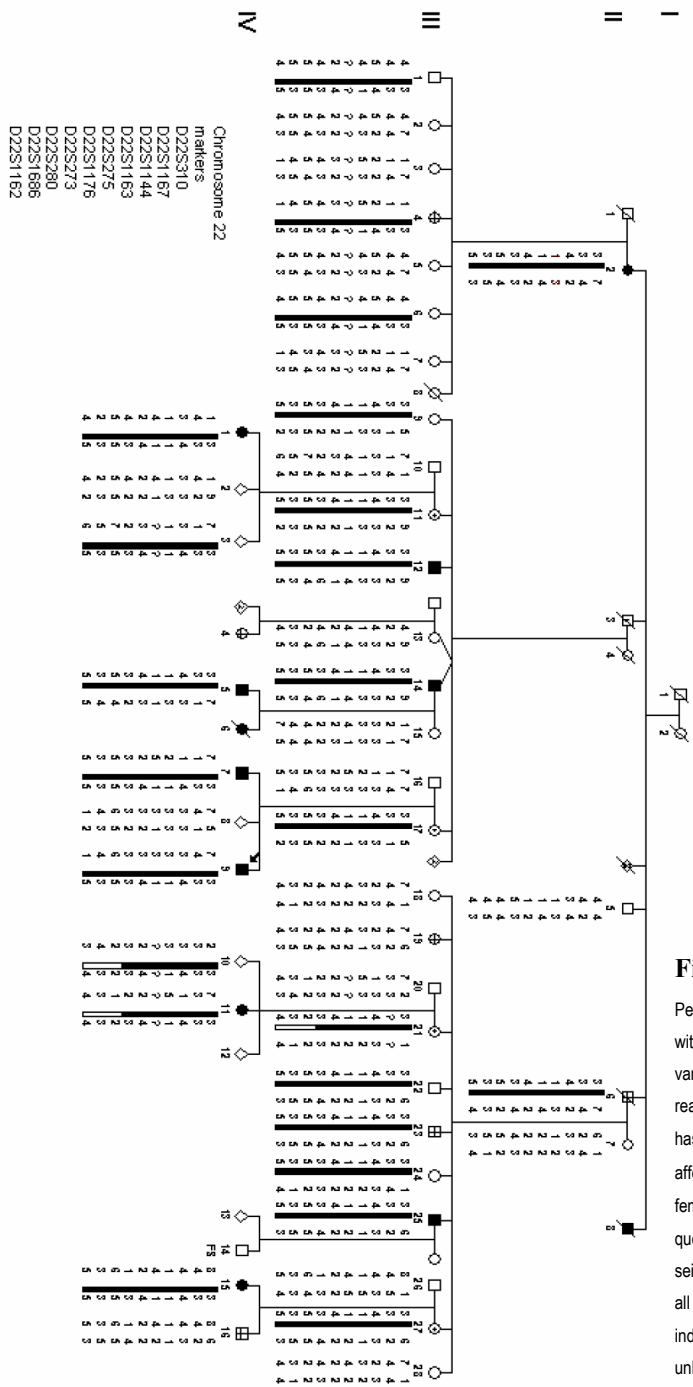


Figure 1

Pedigree of our Dutch family with familial partial epilepsy with variable foci. For privacy reasons, the order of individuals has been changed. ■ = affected male, ● = affected female, • = obligate carrier, + = questionable case, FS = febrile seizures. For linkage analysis, all but the definitely affected individuals were classified as unknown. The proband is indicated by an arrow.

Duration of seizures ranged from 20 seconds to approximately 15 minutes. Seizures were mostly tonic, tonic-clonic or hyperkinetic. They were preceded by autonomic, somatosensory or specific sensory auras in seven patients and accompanied by automatisms in five. None of the patients had auditory symptoms. Seizures occurred in clusters in at least four patients, and could be triggered by stress and sleep deprivation. Intra-individual variation in severity was also observed, with periods of seizures alternating seizure-free periods. Furthermore, seizures were often frequent during childhood and adolescence and tended to decrease in severity and frequency during adulthood although they rarely disappeared completely.

Of one person (III:25), the interictal EEG never showed epileptiform abnormalities; only one of several interictal EEGs of another person (IV:5) showed frontotemporal and frontocentral abnormalities; and half of the interictal EEGs of III:12, III:14, IV:9, IV:11, and IV:15 showed no abnormalities (table 1). In the other interictal EEGs, frontal, central and / or temporal spikes, sharp waves, and spike-wave complexes were observed. An ictal EEG was recorded in two patients, showing frontal and central sharp waves in one (IV:7), and frontotemporal abnormalities in the other (IV:9).

At the time of evaluation, seven patients (aged 12-55 years) still suffered from seizures, of whom six sporadically (< one / month). Nine patients used anti-epileptic drugs, of whom eight were well controlled: one patient had valproic acid monotherapy, one phenobarbital monotherapy, two carbamazepine monotherapy, and five had polytherapy (of whom three had polytherapy with carbamazepine and one with valproic acid).

Table 1

Clinical characteristics of affected members of the Dutch FPEVF family

Patient	Sex	Age (yrs)	Onset (yrs)	Seizure frequency	Current AED	Time of seizure	Seizure classification	EEG findings[a]
II:2	F	88	24	SF 62 yrs	PHB	nocturnal, diurnal	CPS + automatisms, phonatory	frontotemp. slow waves 3-5 Hz
III:12	M	52	24	1-2/year	VPA	nocturnal, diurnal	CPS, SPS speech arrest	50% n.a.; bifrontal epileptic abnormalities (no details)
III:14	M	55	4	1/1-2 mth	CBZ + CLB	nocturnal, diurnal	tonic, CPS + automatisms, SPS	50% n.a.; left frontocentr. waves + spike-wave complexes
III:25	M	47	19	sporadic	CBZ + LTG	shortly after awakening	SPS somatosensory, CPS speech arrest + automatisms	n.a.
IV:1	F	35	3	SF 7 yrs	none	diurnal	CPS, sec. gener. TCS	epileptic abnormalities (no details)
IV:5	M	27	10	sporadic	CBZ + LTG	nocturnal, diurnal	CPS + automatisms	n.a.; 1x frontotemp., frontocentr. parox. slow sharp wave act.
IV:7	M	23	1.8	1/1-3 mth	OXC + LTG	nocturnal, diurnal	SPS posturing of arm(s), CPS TCS	right front., centr., frontotemp., frontocentropar. spikes, spike-wave complexes ictal: right centr., front. sharp waves
IV:9	M	19	0.3	1/night	VPA + LTG	nocturnal, diurnal	CPS +/- automatisms, SPS	50% n.a.; right temp., occ. spikes, waves, polyspike-waves ictal: right frontotemp. 7-8 Hz parox. act.
IV:11	F	12	5	sporadic	CBZ	nocturnal	CPS posturing, SPS somatosensory	50% n.a.; centrotemp. spikes
IV:15	F	22	9.5	SF 22 yrs	CBZ	nocturnal, diurnal	SPS vertiginous, CPS	50% n.a.; prefrontotemp. rare spikes

M = male, F = female, yrs = years, mth = months, SF = seizure free since the age of, AED = anti-epileptic drugs, PHB = phenobarbital, VPA = valproic acid, CBZ = carbamazepine, CLB = clobazam, LTG = lamotrigine, OXC = oxcarbazepine, CPS = complex partial seizures, SPS = simple partial seizures, TCS = tonic-clonic seizures, sec. gener. = secondary generalized, [a] = Except for two EEGs of IV:7 and IV:9, all EEG findings are from interictal EEGs. n.a. = no abnormalities (50% = 50% of the EEGs recorded in this patient showed no abnormalities), temp. = temporal, centr. = central, front. = frontal, par. = parietal, occ. = occipital, parox. act. = paroxysmal activity.

Since no additional EEG studies were performed in these persons, it is unknown whether these periods had an epileptic origin. One person (IV:14) had two febrile seizures at the age of six months, and two others had each a single seizure-like episode of which the epileptic origin could not be confirmed (IV:4, IV:16). For the linkage analysis, the affection status of all these clinically questionable cases was, therefore, regarded as 'unknown'.

Since none of the patients reported auditory or visual symptoms during the seizures, the diagnosis ADLTE was unlikely, despite the fact that in some relatives, including the proband, temporal abnormalities were observed in the interictal EEG. We, therefore, focused our genetic studies on the loci of ADNFLE and FPEVF.

Linkage analysis

Linkage analysis was performed with the three known loci for ADNFLE on chromosome 1p21, 15q24 and 20q13.3, and the two known loci for FPEVF on chromosome 2q36 and 22q11-q12 using several microsatellite markers for each region (D1S498, D1S305 and D1S2635; D15S211, D15S1041, and D15S979; D20S100, D20S443 and D20S171; D2S130, D2S133, and D2S2228; D22S1163 and D22S275). Significantly negative LOD scores (< -2) were found for chromosome 1p21, 2q36, 15q24 and 20q13.3 (data not shown), whereas preliminary evidence for linkage was obtained with the two markers on chromosome 22q11-q12 (multipoint LOD score 2.7). To explore this region further, additional markers were selected from the Marshfield map around D22S1163 and D22S275. The results of haplotype analysis for these markers are shown in figure 1. All affected relatives, ten clinically unaffected relatives and three relatives with vivid dreams and sleepwalking (II:6), nocturnal frightening episodes (III:4), and nightmares (III:23), respectively, carried the disease haplotype (black bar). Of these 13 relatives, five were obligate carriers. Two-point LOD scores between the disease phenotype and each marker are given in table 2.

Table 2

Two-point LOD scores between our family and markers on chromosome 22q11-q12*

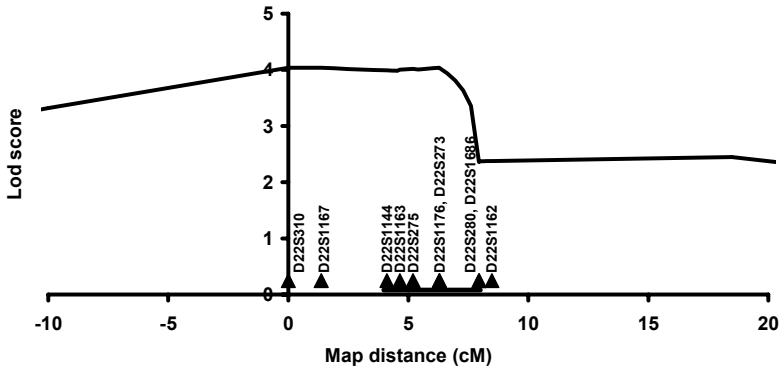
Marker	Location (cM)	Recombination fraction (θ)						Z_{max}	θ_{max}
		0.00	0.01	0.05	0.10	0.15	0.20		
D22S310	18.00	4.037	3.968	3.687	3.319	2.932	2.525	4.037	0.00
D22S1167	19.37	3.822	3.753	3.472	3.104	2.717	2.312	3.822	0.00
D22S1144	22.12	3.276	3.213	2.959	2.630	2.289	1.936	3.276	0.00
D22S1163	22.67	2.262	2.211	2.003	1.738	1.468	1.196	2.262	0.00
D22S275	23.22	1.939	1.897	1.728	1.511	1.291	1.067	1.939	0.00
D22S1176	24.31	3.686	3.617	3.336	2.968	2.582	2.176	3.686	0.00
D22S273	24.31	3.164	3.104	2.860	2.543	2.215	1.874	3.164	0.00
D22S280	25.96	0.916	0.961	1.024	0.981	0.872	0.726	1.024	0.05
D22S1686	25.96	0.952	0.932	0.849	0.745	0.643	0.542	0.952	0.00
D22S1162	26.51	1.898	1.933	1.953	1.855	1.689	1.479	1.960	0.03

Linkage analysis was performed under the assumption of an autosomal dominant mode of inheritance with 50% penetrance, a phenocopy rate of 0.01, and a gene frequency of 0.001. Locations of markers are according to Marshfield. Z_{max} = maximum LOD score for this marker, θ_{max} = recombination fraction at which the maximum LOD score was observed.

Multipoint analysis gave the maximum LOD score of 4.04 at D22S310, D22S1167, and D22S1176 (figure 2). The LOD score dropped to 2.37 at marker D22S280. In person III:21, we observed a haplotype with a recombination between markers D22S273 and D22S280, which was transmitted to her affected daughter (IV:11). On the basis of the LOD scores and the haplotypes, the locus in our family is at least between markers D22S310 and D22S280, a region of 7.93 cM. Since our locus shows complete overlap on the centromeric side with the locus published by Xiong et al. (figure 2, black bar), no additional markers were tested in this region. Therefore, we have no further knowledge of the exact boundary of the disease locus on the centromeric side in our family.

Figure 2

Results of multipoint linkage analysis of our family with chromosome 22q11-q12 markers illustrating the region of linkage. Genetic distances from D22S310 are given in centimorgans. The black bar indicates the region of linkage as described by Xiong et al.[6]



Discussion

We describe a Dutch four-generation family with autosomal dominantly inherited epilepsy with apparent incomplete penetrance. The clinical characteristics of the epilepsy fulfill criteria of both nocturnal frontal lobe epilepsy (ADNFLE) and familial partial epilepsy with variable foci (FPEVF)[2,5,6,10,12,14,32-36]. These syndromes are phenotypically overlapping and, therefore, possibly difficult to differentiate. The most important difference is that patients with FPEVF suffer more frequently from diurnal seizures than patients with ADNFLE, and that EEGs from patients with FPEVF show variable abnormalities, whereas EEGs from patients with ADNFLE predominantly show abnormalities originating from the anterior quadrants[2,5]. Diurnal seizures were, however, also described in families with ADNFLE[36], and EEG abnormalities might also originate from other regions in families with ADNFLE[2,32,35-37]. The frequent occurrence of seizures during daytime, with one of the affected family members (IV:1) suffering from diurnal seizures only, and the observation of interictal EEG abnormalities originating from

different cortical areas are more in agreement with the diagnosis FPEVF in our family. The question why patients with FPEVF have a much more heterogeneous phenotype than patients with ADNFLE has yet to be determined.

Three families with FPEVF have been described until now, one Australian family and two French-Canadian families[5,6]. The French-Canadian families shared an identical linked haplotype and can, therefore, be regarded as one large extended family[6]. Clinical features of the described families were virtually similar to our family: the patients had partial seizures originating from different cortical areas and with variable age at onset. The epileptic focus was frontal or temporal in most patients. Most patients in our family had nocturnal seizures but diurnal seizures were also observed. Since seizures were predominantly nocturnal in the French-Canadian family and mostly diurnal in the Australian family, the clinical characteristics of our family resemble those of the French-Canadian family more closely. The Australian family had suggestive linkage to chromosome 2q36 [5], while the French-Canadian family was linked to chromosome 22q11-q12 [6].

Three affected relatives in our family had psychiatric problems, whereas none of the non-epileptic relatives did. In the Australian family, behavioral problems were described in one affected relative. In the French-Canadian family, four persons with paranoid schizophrenia were identified, but they did not have epilepsy. It was not stated whether these persons had the disease haplotype. Because psychiatric problems were reported in only four out of almost 60 patients in the three families with FPEVF, it seems unlikely that psychiatric disorders are a part of the FPEVF phenotype.

In our family, linkage analysis was performed with the three known ADNFLE loci on chromosome 1p21, 15q24 and 20p13.3, and the two known FPEVF loci on chromosome 2q36 and 22q11-q12. Linkage was observed with

chromosome 22q11-q12, supporting the diagnosis FPEVF. Besides the affected relatives, 13 relatives carried the disease haplotype, including five obligate carriers and three of the four persons with abnormal phenomena during sleep (one of whom, II:6, was obligate carrier). The question, therefore, arises whether these phenomena might be of epileptic origin. Three of the other clinically unaffected relatives that carried the haplotype (III:22, IV:3, IV:10) were younger than 45 years and may still be at risk of developing epilepsy at a later age.

Our family is the first family that confirms linkage of FPEVF to chromosome 22q11-q12, previously reported by Xiong et al.[6] We observed a recombination between markers D22S273 and D22S280 in person III:21 that was transmitted to her affected daughter (IV:11), indicating the telomeric boundary of the locus in our family. This end is at the same marker as observed by Xiong et al. The boundary of the disease locus on the centromeric side in our family is unknown but our region of linkage on this side is larger than that in the published French-Canadian family[6]. The linked region, therefore, overlaps their region of linkage; we were unable to reduce the area.

The candidate region of at least 7.93 cM defined by haplotype reconstruction and linkage analysis has a high density of known and putative genes (more than 100) and is physically large (approximately 6.5 Mb) (Genemap; www.ncbi.nlm.nih.gov/genemap99). Candidate genes in this area are the seizure related gene 6 homolog-like (SEZ6L) and the genes encoding synapsin III and tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein (YWHAH). In humans, the SEZ6L gene on chromosome 22q11-q12 is quite similar to the seizure related 6 homolog gene (SEZ6) on chromosome 17q11.2, which encodes a brain-specific membrane protein. In mice, *Sez6* is located on chromosome 11B5 [38]. It was identified with linkage analysis in mice, showing tonic-clonic seizures after pentylenetetrazol injection[38]. Pentylenetetrazol acts as a convulsant via the $GABA_A$ benzodiazepine-receptor

complex. By determining the minimal dose to induce convulsions in mice, a good estimate of the general excitability of the central nervous system can be obtained. Synapsin III is a neuron-specific synaptic vesicle-associated phosphoprotein, involved in the regulation of neurotransmitter release and synaptogenesis[39]. Knockout mice for synapsin I and/or II experience seizures with a frequency proportional to the number of mutant alleles[40]. D22S280 is located within an intron between exons 6 and 7 of the synapsin III gene. YWHAH encodes a protein controlling intracellular signaling and neurotransmitter release[41]. The protein is located exclusively in the cytoplasm of neurons in the cerebral cortex. This protein may be associated with neuropsychiatric disorders[42]. Whether one of these genes is involved in the epilepsy of this family will have to be determined by sequence analysis.

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