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Autosomal Dominant Nocturnal Frontal-Lobe Epilepsy: Genetic Heterogeneity and Evidence for a Second Locus at 15q24

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Summary

Autosomal dominant nocturnal frontal-lobe epilepsy (ADNFLE) is a recently identified partial epilepsy in which two different mutations have been described in the α4 subunit of the neuronal nicotinic acetylcholine receptor (*CHRNA4*). An additional seven families are presented in which *ADNFLE* is unlinked to the *CHRNA4* region on chromosome 20q13.2. Seven additional sporadic cases showed no evidence of defective *CHRNA4*. One of the families showed evidence of linkage to 15q24, close to the *CHRNA3/CHRNA5/CHRNB4* cluster (maximum LOD score of 3.01 with *D15S152*). Recombination between *ADNFLE* and *CHRNA4*, linkage to 15q24 in one family, and exclusion from 15q24 and 20q13.2 in others demonstrate genetic heterogeneity with at least three different genes for ADNFLE. The *CHRNA4* gene and the two known *CHRNA4* mutations are responsible for only a minority of ADNFLE. Although the ADNFLE phenotype is clinically homogeneous, there appear to be a variety of molecular defects responsible for this disorder, which will provide a challenge to the understanding of the basic mechanism of epileptogenesis.

Introduction

Autosomal dominant nocturnal frontal-lobe epilepsy (ADNFLE; MIM 600513) is a recently identified idiopathic partial epilepsy (Scheffer et al. 1994, 1995). This epilepsy syndrome is characterized by clusters of frontal-lobe motor seizures occurring during sleep. Onset is usually in childhood, and inheritance is autosomal dominant, with 75% penetrance. The condition is clinically distinctive and relatively homogeneous, although seizure severity and specific frontal-lobe seizure manifestations vary within families (Scheffer et al. 1995; Hayman et al. 1997). Misdiagnosis as nightmares, night terrors, other parasomnias, or even psychiatric disorders is common if clinicians are unaware of ADNFLE.

The gene for ADNFLE in one large Australian family was mapped to 20q13.2 (Phillips et al. 1995). In this family the molecular defect was found to be a C→T transition that replaces serine by phenylalanine at codon 248 in the α4 subunit of the neuronal nicotinic acetylcholine receptor (CHRNA4) (Steinlein et al. 1995). The molecular defect of the same gene in a second ADNFLE family, of Norwegian origin, has been characterized as an insertion of a GCT triplet between codons 259 and 260 (Steinlein et al. 1997). Thus far, these are the only two mutations detected for *ADNFLE*, and both have been shown to have major effects on receptor function in vitro (Weiland et al.1996; Kuryatov et al. 1997; Steinlein et al. 1997).

Several genes for other idiopathic epilepsies have been mapped. These include benign familial neonatal convulsions (20q13.2 and 8q) (Leppert et al. 1989; Lewis et al. 1993), partial epilepsy with auditory symptoms (10q22-q24) (Ottman et al. 1995), familial febrile seizures (8q13-q21 and 19p13.3) (Wallace et al. 1996; Johnson et al. 1998), and benign familial infantile convulsions (19q and 16) (Guipponi et al. 1997; Szepetowski et al. 1997). Thus, at least three of these monogenic epilepsies demonstrate locus heterogeneity. For benign neonatal familial convulsions, the only idiopathic epilepsy (apart from ADNFLE) in which genetic defects have been identified, the two genes code for homologous potassium channels (Biervert et al. 1998; Charlier et al. 1998; Singh et al. 1998).

Our preliminary studies of additional families with ADNFLE suggest that they do not show linkage to the CHRNA4 locus at 20q13.2 (Berkovic et al. 1995). There are several neuronal nicotinic acetylcholine–receptor subunits (α2–α9 and β2–β4), and each functional re-

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ceptor is composed of five subunits; the most common functional receptor in brain is thought to be composed of two $\alpha 4$ subunits and three $\beta 2$ subunits (Schoepfer et al. 1988; Whiting et al. 1991; Sargent 1993). Given the involvement of *CHRNA4* in ADNFLE, the other neuronal nicotinic acetylcholine–receptor subunits have emerged as candidates for ADNFLE and, possibly, other idiopathic epilepsies (Elmslie et al. 1997) in families which do not map to *CHRNA4*. Known locations of other subunits expressed in brain are 1p21 ($\beta 2$), 8p11.2 ($\beta 3$), 8p21 ($\alpha 2$), 15q13 ($\alpha 7$), and 15q24 ($\alpha 3$, $\alpha 5$, and $\beta 4$).

We now show that the two reported *CHRNA4* mutations are uncommon among patients with familial or sporadic nocturnal frontal-lobe epilepsy. We also show that seven ADNFLE families are not linked to the *CHRNA4* locus, and, in one of these families, we find linkage to markers on chromosome 15, near the *CHRNA3/CHRNA5/CHRNB4* cluster.

Subjects and Methods

Subjects

A. Familial cases.—Multigenerational families in which DNA was available from at least four individuals affected with ADNFLE were selected for study (fig. 1). The probands had idiopathic nocturnal frontal-lobe epilepsy with the characteristics defined elsewhere (Scheffer et al. 1995), and affected relatives had similar clinical patterns with variable severity. Families C, D, G, and O have not been reported elsewhere and are of Italian, Scottish, French Canadian, and Swedish origin, respectively. Families S (Australian) and M (English) were reported, in part, as pedigrees B and C, respectively, in Scheffer et al. (1995). These two pedigrees have been considerably extended, with new affected individuals having been identified. Family Q is of French Canadian origin and has been reported elsewhere (Lopes-Cendes et al. 1995; Scheffer et al. 1995).

B. Sporadic Cases.—Subjects with idiopathic nocturnal frontal-lobe epilepsy and a negative family history of seizures, despite careful inquiry, were studied. Inclusion criteria were onset of seizures at age <25 years, partial seizures with frontal-lobe semiology, $\geqslant 90\%$ of seizures occurring during sleep, normal intellect, normal neurological examination, and normal magnetic-resonance imaging. Magnetic-resonance scans were carefully scrutinized, to exclude subtle dysplastic abnormalities. There were seven Australian subjects (four females) with seizure onset at age 2–21 years (mean 8 years).

Mutation analysis

All families and sporadic cases were tested for the two known mutations in *CHRNA4* (Steinlein et al. 1995, 1997), by single-strand conformation analysis (SSCA) and direct DNA sequencing using primers 8 and 11 (table 1). They were screened for novel mutations by SSCA within exons 2 and 4–6 and by sequencing across the transmembrane domains I–III. Exon 3 was too small (55 bp), and exon 1 was too GC rich, for easy analysis, and, since exons 5 and 6 contain the main coding region, which includes the four conserved transmembrane domains and the sites of the two known mutations, exons 1 and 3 were not examined. Primers used for SSCA and sequencing of *CHRNA4* are shown in table 1. These were designed directly from the cDNA sequence (GenBank accession number U62433), by means of the chicken *CHRNA4* genomic organization (GenBank accession numbers X07348–X07352 and X07399), to infer likely locations of intron-exon boundaries. Exons 5 and 6 of *CHRN* subunits $\beta 2$, $\beta 4$, $\alpha 3$, and $\alpha 5$ were analyzed by SSCA (primers are available from the corresponding author, on request). All SSCA variants were sequenced to determine the molecular basis of the variation.

PCR reactions for SSCA were performed on 100 ng of genomic DNA in a total volume of 10 μl containing 1.5 mM $MgCl_2$, 2 μCi of [^{32}P]-dCTP, and 150 ng of each primer. Cycling was at 94°C for 1 min, 60°C for 1.5 min, and 72°C for 1.5 min (10 cycles) and at 94°C for 1 min, 55°C for 1.5 min, and 72°C for 1.5 min (25 cycles), followed by 10 min at 72°C, in a Perkin-Elmer thermocycler. PCR products were run on 6% and 10% nondenaturing polyacrylamide gels containing 5% glycerol and on MDE gels (FMC BioProducts), according to the manufacturer's instructions. DNA sequencing was performed by means of a Perkin-Elmer Ready Reaction Dye Terminator Kit, in a Perkin-Elmer thermocycler, with 25 ng of primer and ~70 ng of double-stranded PCR product as template.

Linkage Analysis

Observations of recombination between *ADNFLE* and intragenic *CHRNA4* markers were used to exclude linkage of *ADNFLE* to *CHRNA4* in the seven families. The intragenic *CHRNA4* polymorphisms described elsewhere consist of three *Cfo*I polymorphisms, at positions 594 (Steinlein 1995), 1545 (Phillips and Mulley 1997), and 1575 (Phillips and Mulley 1997) and an AC dinucleotide-repeat polymorphism in intron 1 (Weiland and Steinlein 1996). A family-specific intragenic *CHRNA4* variant detected by SSCA and sequencing (see Results) was used to exclude linkage to *CHRNA4* in family S.

LOD scores, between *ADNFLE* and chromosome 15 markers from the Généthon linkage map (Dib et al. 1996), were calculated by MLINK (Lathrop and Lalouel 1984). Only affected family members were used to ex-

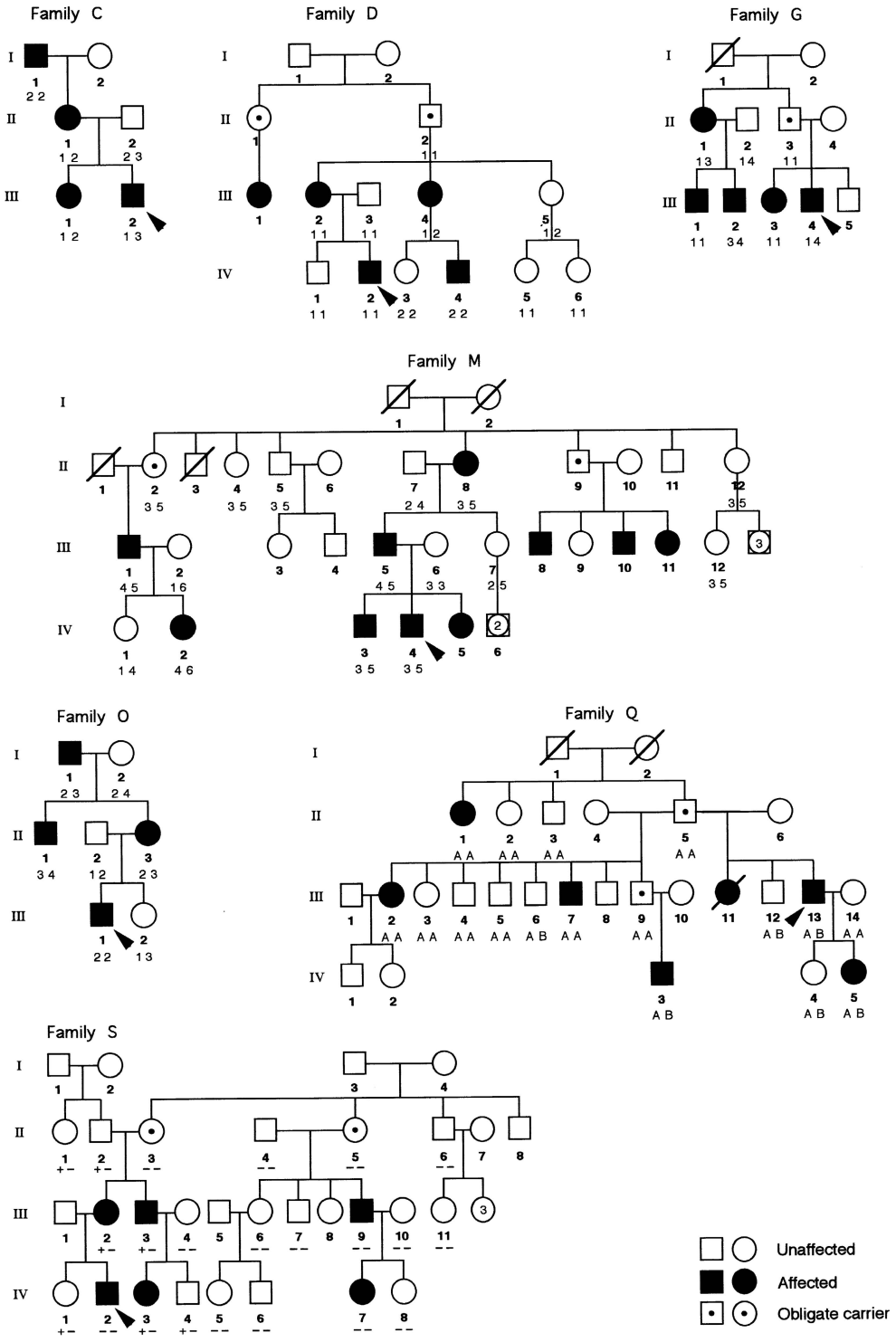

Figure 1 Families studied. Markers within *CHRNA4* used to show recombination with *ADNFLE* are the AC dinucleotide repeat in intron 1 (Weiland and Steinlein 1996) for families C, D, G, M and O, the *Cfo*I polymorphism at 594 (Steinlein 1995) for family Q and the private variant described in the text for family S.

Table 1

Primer Pairs Used in SSCA and Sequencing of *CHRNA4*

A. Exons and Primer Pairs Used	
Exon(s)	Primer Pair(s)
2	1-2
4	3-4
5	5-7, 5-9, 5-10; 6-9, 6-10[a]; 8-10, 8-11[a]; 12-14; 13-14, 13-17; 15-17, 15-20[a]; 16-20; 18-20
5 and 6	19-22
6	21-22[a]

B. Primer Sequences	
Primer	Sequence
1	ACGCCGAGGAGCGGCT
2	CACGTCAATGAGCTGAG
3	GAGTGGCACGACTACAAGCT
4	TTGTTGTAGAGGACGATGTCCGGC
5	CGGCCATTTACAAGAGCTCC
6	GGCGAGTGGGTCATCGTGG
7	TAGGTGATGTCCGGGTAGAT
8	TCACCTATGCCTTCGTCATC
9	GGTGAGCGACAGCAGCAC
10	ATGACCAGTGAGGTGGACG
11	TGGCACGATGTCCAGGAAGA
12	TCTTCCTGGACATCGTGCCA
13	TTCCTGCAAGTCACCCT
14	GGGAACACAGTACTGGAT
15	ATCCAGTACTGTGTTCCC
16	AAGGAGCCCTCTTCGGTGTC
17	TTTGGTGCTGCGGGTCTTGA
18	TCAAGACCCGCAGCACCAAA
19	GTCCAGTACATTGCAGACCA
20	CGAGAAGTCTGTGTCTTC
21	GTGAAGGAGGACTGGAAGTA
22	TCCCTTCCTAGATCATGCCA

[a] Primer pair also was used for sequencing of both DNA strands of PCR product.

clude linkage, by recombination, and to demonstrate linkage, by LOD-score analysis, since penetrance may vary, depending on the gene or mutation involved. The locations of *CHRNA3, CHRNA5,* and *CHRNB4* were determined in relation to Généthon markers on chromosome 15, by means of the Stanford G3 radiation-hybrid panel (Stewart et al. 1997).

Results

CHRNA4

None of the seven families or any of the sporadic cases had either of the two known mutations, nor did they have any other mutation detectable by SSCA, in the parts of the gene analyzed. Sequencing of those parts of the gene containing transmembrane domains confirmed this result. Mutation analysis did, however, reveal a number of benign polymorphisms, as described above (Subjects and Methods), and a rare variant, in exon 5, peculiar to family S (not seen in 129 unrelated individuals) when primer pair 8-11 was used (table 1). This is a nonconservative T→G transition, which effects an isoleucine-to-serine change in the conserved transmembrane I domain, at codon 229, according to the sequence nomenclature of Steinlein et al. (1996), or codon 257, according to the sequence given by GenBank. This variant shows Mendelian inheritance but does not show 1:1 segregation with the ADNFLE in family S (fig. 1).

Linkage analysis using intragenic polymorphisms described above (Subjects and Methods) and an intragenic marker detected, in this study, in family S showed that ADNFLE in these families is not due to mutation of *CHRNA4* (fig. 1). In family C, neither III-1 nor III-2 received a copy of *CHRNA4* from the affected grandfather (I-2). In family D, affected individuals IV-2 and IV-4 received different copies of *CHRNA4*. In family G, affected individuals III-1 and III-2 inherited different *CHRNA4* alleles from their affected mother (II-1). In family M, affected individual IV-2 did not inherit a copy of *CHRNA4* from her carrier grandmother (II-2). In family O, affected individual III-1 did not receive a copy of the gene from his grandfather (I-1). In family Q, affected individual IV-5 did not inherit a copy of *CHRNA4* from her nonpenetrant grandfather (II-5). In family S, the affected individual IV-3 did not inherit a copy of *CHRNA4* from her obligate-carrier grandmother (II-3), as determined by the segregation of the rare variant specific to this family. Thus, recombination between *ADNFLE* and *CHRNA4* in affected members in all of these families excludes linkage to *CHRNA4* and, hence, demonstrates genetic heterogeneity for ADNFLE.

CHRNB2

SSCA analysis of transmembrane-domain regions of exons 5 and 6, in all families and sporadic cases, failed to detect any band shift. Linkage analysis using intragenic *CHRNB2* markers was not possible, since no polymorphisms were observed by SSCA.

CHRNA3, CHRNA5, *and* CHRNB4

Généthon markers from the chromosome 15 region containing *CHRNA3, CHRNA5,* and *CHRNB4* were used to look for linkage in the seven ADNFLE families. The largest family (family M) showed linkage to chromosome 15 (table 2). The maximum LOD score was 3.01 at recombination fraction of 0, with the fully informative marker *D15S152*. The haplotype of markers in family M that is consistent with linkage of *ADNFLE* to the long arm of chromosome 15 is given in figure 2. The regional localization as determined from data for family M is between *D15S211* (proximal recombination in two affected individuals IV-3 and IV-4) and *D15S979* (distal recombination in one affected individual, III-10) (fig. 2). Linkage to 15q24 was excluded in four families

Table 2

Pairwise Lod Scores between ADNFLE and Informative Chromosome 15 Markers Mapping between *D15S211* and *D15S979*, for Families C, D, G, M, O, Q, and S

Family and Marker	LOD Score at Recombination Fraction of						
	.00	.01	.05	.1	.2	.3	.4
C:							
D15S211	.60	.59	.56	.51	.41	.29	.16
D15S1041	.30	.29	.26	.21	.13	.06	.02
D15S206	.60	.59	.56	.51	.41	.29	.16
D15S205	.30	.29	.26	.21	.13	.06	.02
D15S152	.60	.59	.56	.51	.41	.29	.16
D15S201	.60	.59	.56	.51	.41	.29	.16
D15S151	.60	.59	.56	.51	.41	.29	.16
D15S1030	.60	.59	.56	.51	.41	.29	.16
D15S979	.60	.59	.56	.51	.41	.29	.16
D:							
D15S211	1.37	1.34	1.23	1.08	.77	.47	.21
D15S1041	1.28	1.26	1.15	1.01	.73	.45	.20
D15S206	.30	.28	.20	.11	−.02	−.07	−.05
D15S205	.78	.76	.70	.62	.47	.32	.16
D15S152	.76	.74	.66	.57	.39	.23	.10
D15S201	.48	.47	.42	.37	.26	.17	.08
D15S151	.38	.37	.34	.30	.23	.16	.08
D15S1030	.95	.92	.82	.69	.44	.24	.10
D15S979	1.28	1.26	1.15	1.01	.73	.45	.20
G:							
D15S211	−2.79	−.90	−.29	−.08	.03	.03	.01
D15S1041	.84	.82	.73	.63	.42	.22	.06
D15S200	−2.81	−.92	−.31	−.10	.02	.02	.01
D15S979	−2.81	−.92	−.31	−.10	.02	.02	.01
M:							
D15S211	−1.69	−.96	.19	.55	.65	.45	.16
D15S1041	2.28	2.23	2.02	1.76	1.20	.65	.18
D15S206	2.16	2.12	1.95	1.72	1.27	.79	.34
D15S205	2.41	2.36	2.19	1.96	1.47	.94	.40
D15S152	3.01	2.96	2.74	2.46	1.85	1.19	.51
D15S201	2.01	1.97	1.82	1.63	1.22	.79	.37
D15S151	1.22	1.19	1.09	.96	.71	.47	.27
D15S1030	1.08	1.05	.94	.79	.53	.29	.12
D15S979	−.95	.97	1.46	1.51	1.52	.83	.38
O:							
D15S1041	−3.80	−1.40	−.74	−.48	−.26	−.16	−.08
D15S205	.30	.29	.26	.21	.13	.06	.02
D15S152	−3.80	−1.70	−1.00	−.70	−.40	−.22	−.10
D15S201	−3.80	−1.70	−1.00	−.70	−.40	−.22	−.10
D15S979	−3.80	−1.40	−.74	−.48	−.26	−.16	−.08
Q:							
D15S1041	−3.07	−.88	−.26	−.06	.04	.04	.01
D15S205	−6.35	−3.65	−1.94	−1.15	−.47	−.18	−.04
D15S152	−2.80	−2.24	−1.06	−.57	−.19	−.05	−.01
D15S201	.32	.32	.31	.29	.23	.16	.08
D15S979	−3.08	−.89	−.27	−.08	.01	−.01	−.04
S:							
D15S1041	−3.40	−2.53	−1.26	−.74	−.30	−.11	−.02
D15S205	−3.40	−2.78	−1.55	−1.03	−.55	−.29	−.11
D15S152	−3.07	−1.03	−.39	−.17	−.01	−.03	.02
D15S201	−3.10	−2.86	−1.83	−1.27	−.72	−.41	−.19
D15S979	−3.10	−3.10	−2.39	−1.62	−1.85	−.45	−.19

(families G, O, Q and S), by recombination (table 2). In families C and D, there were no recombinants between *ADNFLE* and markers from 15q24, but the families were too small to be used to establish linkage (fig. 2 and table 2).

Results of radiation-hybrid–panel mapping indicated that *D15S1227* was the marker closest to the three candidate genes *CHRNA3, CHRNA5,* and *CHRNB4,* which places them between *D15S114* and *D15S1041* on the Généthon map (fig. 3). Since *D15S211* (the marker delineating the proximal limit for the *ADNFLE* regional localization) also maps between *D15S114* and *D15S1041,* these candidate genes must map to the 1.6-cM interval between *D15S211* and *D15S1041,* if any of them are responsible for ADNFLE in family M.

SSCA of transmembrane-domain regions in exons 5 and 6 of *CHRNA3* and *CHRNA5* failed to detect any band shift. However, sequencing revealed polymorphisms (not detected by SSCA) in exon 5 of *CHRNA3* and in exon 5 of *CHRNA5*.

The *CHRNA3* polymorphism is a conservative T→C change at base 708 (GenBank accession number M37981). Allele frequencies are as follows: .56 for the C allele and .44 for the T allele, on the basis of data on 18 unrelated chromosomes. A mismatch primer was designed to create an *Rsa*I site in the allele carrying the C at this position, so that the polymorphism could be detected by PCR using primers A (5′-ATC ATC AAA GCC CCA GGG TA-3′) and B (5′-AGT GAG GAA GGA GAT GAG CA-3′), followed by digestion of the PCR product by *Rsa*I. This gave fragments of (*a*) 37 bp, 45 bp, and 71 bp for the T allele and (*b*) 18 bp, 19 bp, 45 bp, and 71 bp, for the C allele.

The *CHRNA5* polymorphism is a conservative G→A change at base 1340 (GenBank accession number M83712), which destroys a *Taq*I site. PCR amplification with primers A (5′-TCA TCG TTC TTC CTC AAC AC-3′) and B (5′-CGG ACA TCA TTT TCC TTC AT-3′), followed by digestion with *Taq*I, gives fragments of (*a*) 228 bp for the A allele and (*b*) 177 bp and 51 bp for the G allele. Allele frequencies are as follows: .28 for the A allele and .72 for the G allele, on the basis of data on 54 unrelated chromosomes.

SSCA of exons 5 and 6 of *CHRNB4* detected a band shift in exon 5, with primers A (5′-ACG GGA CCT ATG AGG TGT CT-3′) and B (5′-GGG AGT AAA GTC ATC CAT GC-3′). Sequencing revealed that this was due to a conservative C→T change at base 312 (GenBank accession number X68275), which destroys a *Cfo*I site. Digestion of the PCR product by *Cfo*I gives fragments of (*a*) 227 bp for the T allele and (*b*) 90 bp and 137 bp for the C allele. Allele frequencies are as follows: .86 for the C allele and .14 for the T allele, on the basis of data on 66 unrelated chromosomes.

These polymorphisms could be useful for exclusion

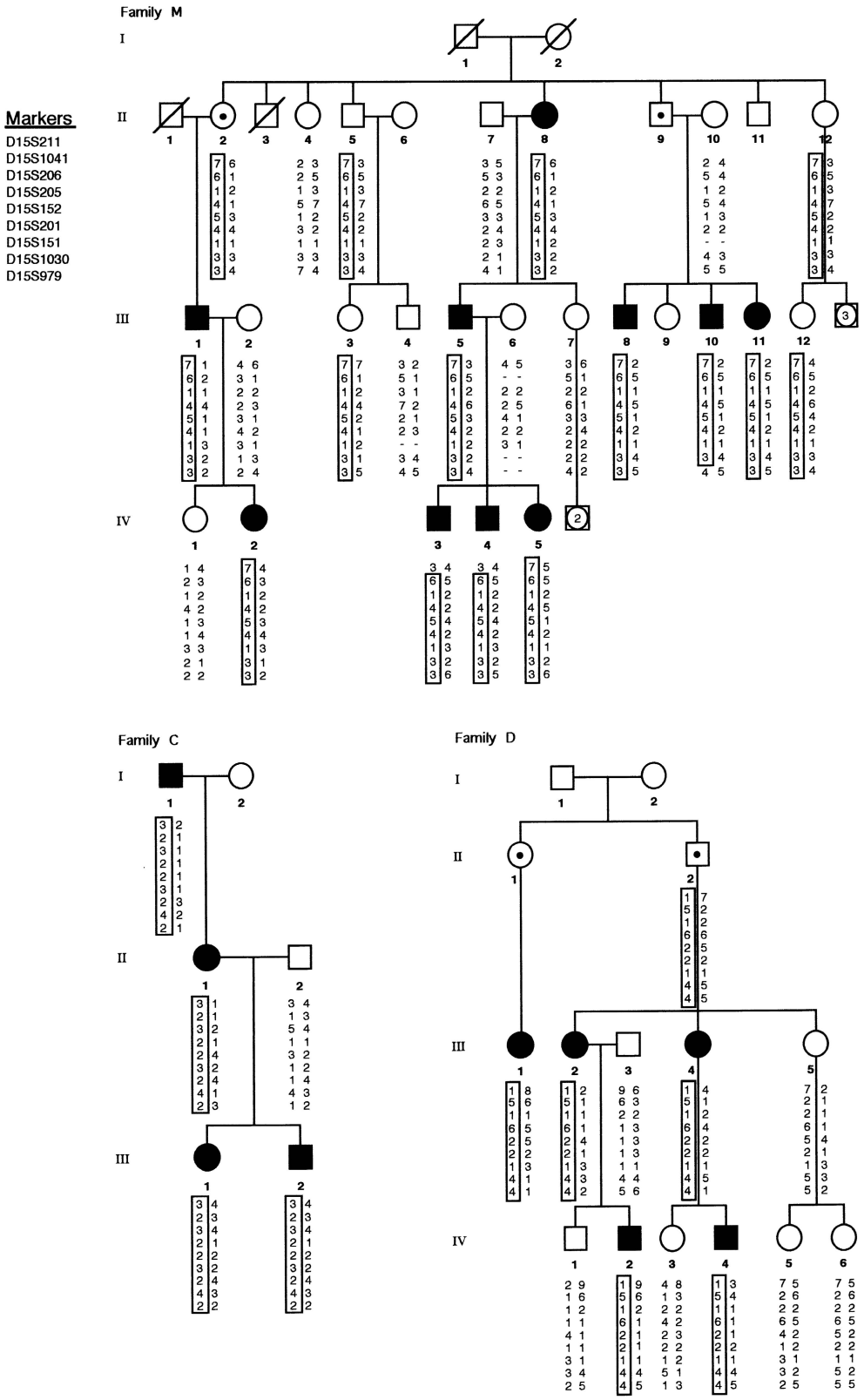

Figure 2 Segregation of haplotypes in the three families consistent with linkage of *ADNFLE* to chromosome 15

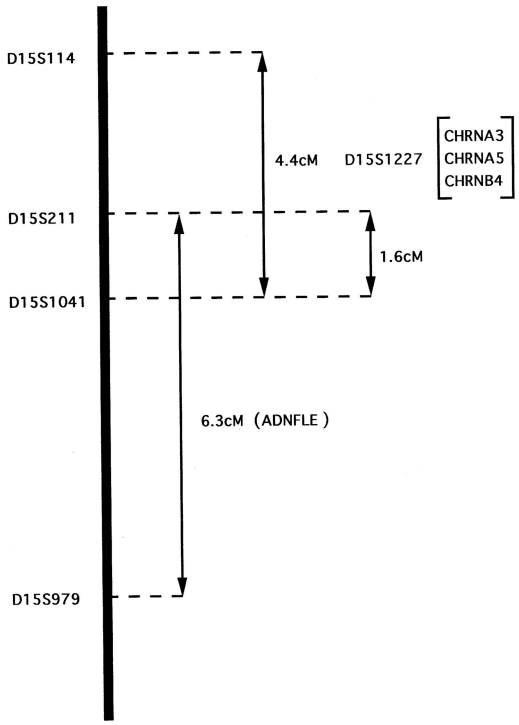

Figure 3 Généthon linkage map of part of chromosome 15 in proximity to *CHRNA3, CHRNA5* and *CHRNB4*.

(by recombination) of the *CHRNA3, CHRNA5,* or *CHRNB4* genes as a cause of ADNFLE—or of any other disorder for which they are candidates. They are applicable to exclusion analysis of families too small to provide significant LOD scores by linkage analysis. None of these polymorphisms are fully informative in families M, C, and D, and, since no recombination with *ADNFLE* was observed, these genes remain candidates for ADNFLE.

Discussion

ADNFLE is the prototype of a newly recognized group of human partial epilepsies with monogenic inheritance (Berkovic and Scheffer 1997). Localization of the gene for ADNFLE in a large Australian family (Phillips et al. 1995) led to the identification of *CHRNA4* as the first gene to be implicated in a human idiopathic epilepsy (Steinlein et al. 1995, 1997). Apart from two families in which *CHRNA4* mutations have been found, no other mutations have been reported in other ADNFLE families.

The distinctive clinical pattern of clusters of nocturnal motor seizures is characteristic of epilepsy arising in the frontal lobe. This phenotype can be observed in sporadic cases with known frontal-lobe lesions such as tumors, trauma, dysplasia, etc. Epileptologists also often observe sporadic cases with no evidence, on magnetic-resonance scans, of a structural lesion and with no history of brain insult; it is unknown whether such cases have occult lesions or are new mutations giving rise to ADNFLE. Here we have shown that the two known *CHRNA4* mutations are absent in seven other families with ADNFLE and in seven sporadic cases, suggesting that these mutations are uncommon or rare causes of ADNFLE. No other *CHRNA4* mutations were found in the seven sporadic cases. The use of intragenic *CHRNA4* markers excluded linkage to *CHRNA4* in the seven families (fig. 1), thus demonstrating genetic heterogeneity of this disorder, which is in agreement with recent findings of Oldani et al. (1998).

The only possibly significant abnormality in our analysis of *CHRNA4* was the rare variant in family S. It is a nonconservative change (I257S) in the conserved transmembrane I domain. This domain is believed to form part of the ion channel, and certain amino acid changes in the transmembrane I domain of the muscle acetylcholine α–receptor subunit (N217K) and the transmembrane I domain of the muscle acetylcholine ϵ subunit (P245L) have been shown to cause slow-channel myasthenic syndrome, by slowing the rate of channel closing (Engel et al. 1996; Ohno et al. 1997). The relative position of *CHRNA4* codon 257 (according to the nomenclature of GenBank accession number U62433) is midway between these two. However, the mutation in family S originates from the grandpaternal side of the family whereas ADNFLE originates from the grandmaternal side. Possible explanations could be that (1) the amino acid involved may not face into the channel opening and therefore has little or no effect on ion flow, (2) this variant is part of the genetic background, acting as a modifier to lower the seizure threshold, or (3) there are two genes for ADNFLE that are segregating in this family, one of which is *CHRNA4* with a defective codon 257.

The exclusion of seven families from the *CHRNA4* region, the localization of one family to chromosome 15, and, in other families, the exclusion of linkage to chromosome 15 demonstrates the existence of at least three genes responsible for ADNFLE. Some families with this epilepsy syndrome do not show evidence of linkage either to *CHRNA4* (fig. 1; also see Oldani et al. 1998) or near the *CHRNA3/CHRNA5/CHRNB4* cluster on chromosome 15 (table 2; also see Eng et al. 1991; Lopes-Cendes et al. 1995). Families C and D, in which linkage to 15q could not be excluded, and family M were not included in the study by Lopes-Cendes et al. (1995), which originally excluded *ADNFLE* from this region.

Mutation analysis of the transmembrane domains in exons 5 and 6 of *CHRNA3, CHRNA5,* and *CHRNB4* failed to detect any mutation. Although these genes re-

main good candidates, the regional localization of *ADNFLE* on chromosome 15 is ~6.3 cM, and the possibility that a gene other than *CHRNA3, CHRNA5,* or *CHRNB4* is responsible for ADNFLE must not be overlooked. The three subunits *CHRNA3, CHRNA5,* and *CHRNB4* are likely to be close to each other, given that they are clustered within a 68-kb region in the rat genome (Boulter et al. 1990). Radiation-hybrid mapping confirmed their close proximity in humans (fig. 3). Other possible candidate genes in this region include the neurotrophic tyrosine kinase receptor, type 3 (*NTRK3*), which is preferentially expressed in brain and is thought to be important in the development of certain areas of the CNS, and neuromedin B (*NMB*), a bombesin-like peptide widely distributed in mammalian neural and endocrine cells (OMIM; also see Pearson et al. 1994).

Families G, O, Q, and S have ADNFLE that is not due to mutation in *CHRNA4,* and they show no suggestion of linkage to 15q24. In family Q, linkage has been excluded in this region (Lopes-Cendes et al. 1995), which is one of the possible human chromosomal regions homologous to the mouse region containing the El-1 mouse locus; the El mouse has spontaneous partial seizures inherited in a complex manner (Rise et al. 1991). Other neuronal nicotinic acetylcholine–receptor subunits are potential candidates as the cause of epilepsy in these families. *CHRNB2* on chromosome 1 is a prime candidate, since it combines with *CHRNA4* to form the most common nicotinic acetylcholine receptor in the brain (Schoepfer et al. 1988; Whiting et al. 1991; Sargent 1993). SSCA of exons 5 and 6 containing transmembrane domains did not detect any mutation of *CHRNB2* in affected individuals; however, SSCA does not detect all mutations (Hayashi and Yandell 1993).

The findings presented here show that there are at least three loci for ADNFLE. Pharmacological studies of the two known *CHRNA4* mutations have shown surprisingly different effects in vitro, although both mutations appear to impair calcium entry into neurons (Weiland et al. 1996; Kuryatov et al. 1997; Steinlein et al. 1997). Thus, it appears that a variety of basic molecular mechanisms can lead to the phenotype of ADNFLE, which is clinically relatively homogeneous. This molecular heterogeneity will provide a challenge to the designing of new rational therapies for ADNFLE. However, the hypothesis that fundamentally the phenotype is due to impaired presynaptic nicotinic cholinergic transmission in the frontal lobes remains attractive and is consistent with current molecular genetic and pharmacological data.

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Electronic-Database Information

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

GenBank, http://www.ncbi.nlm.nih.gov/irx/cgi-bin/genbank (for *CHRNA4* codon 257 [U62433], chicken *CHRNA4* [X07348–X07352 and X07399], *CHRNA3* T→C at position 708 [M37981],*CHRNA5* G→A at position 1340 [M83712], and *CHRNB4* C→T at position 312 [X68275])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov (for ADNFLE [MIM 600513])

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