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Childhood Absence Epilepsy with Tonic-Clonic Seizures and Electroencephalogram 3–4-Hz Spike and Multispike–Slow Wave Complexes: Linkage to Chromosome 8q24

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Summary

Childhood absence epilepsy (CAE), a common form of idiopathic generalized epilepsy, accounts for 5%–15% of childhood epilepsies. To map the chromosomal locus of persisting CAE, we studied the clinical and electroencephalographic traits of 78 members of a five-generation family from Bombay, India. The model-free affected–pedigree member method was used during initial screening with chromosome 6p, 8q, and 1p microsatellites, and only individuals with absence seizures and/or electroencephalogram 3–4-Hz spike– and multispike–slow wave complexes were considered to be affected. Significant *P* values of .00000–.02 for several markers on 8q were obtained. Two-point linkage analysis, assuming autosomal dominant inheritance with 50% penetrance, yielded a maximum LOD score (Z_{max}) of 3.6 for D8S502. No other locus in the genome achieved a significant Z_{max}. For five smaller multiplex families, summed Z_{max} was 2.4 for D8S537 and 1.7 for D8S1761. Haplotypes composed of the same 8q24 microsatellites segregated with affected members of the large family from India and with all five smaller families. Recombinations positioned the CAE gene in a 3.2-cM interval.

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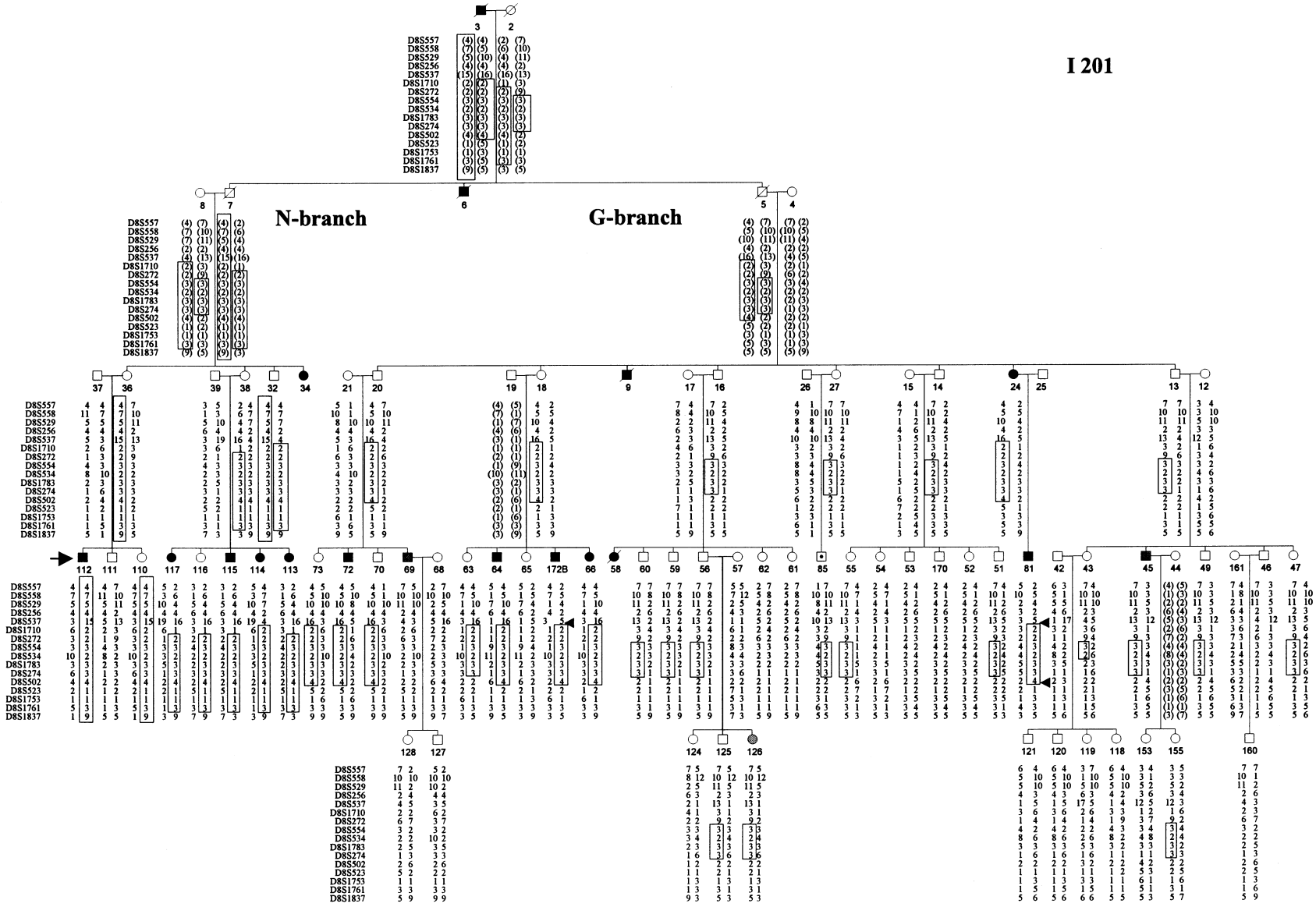
Introduction

Idiopathic generalized epilepsies are common neurological disorders, accounting for ~39%–59% of the 3 million to 4 million Americans suffering from epilepsy (Hauser and Hesdorffer 1990). Juvenile myoclonic epilepsy, childhood absence epilepsy (CAE), and epilepsy with tonic-clonic seizures on awakening are estimated to account for most of the genetically determined idiopathic generalized epilepsies. CAEs with 3-Hz spike and wave complexes on electroencephalograms (EEGs) are conservatively estimated to account for at least 2.3%–37.7% of all epilepsies (Lennox 1945; Livingston et al. 1965) and 8%–15% of all childhood epilepsies in school-aged children (Cavazzuti 1980). There are at least three subsyndromes of CAE. The first subsyndrome, which accounts for ~40%–60% of CAE patients, is characterized by absence seizures as the sole phenotype and remits spontaneously during adolescence. The second subsyndrome, which accounts for another 40% of CAE patients, persists into adolescence and adulthood, during which patients develop tonic-clonic seizures. The third subsyndrome accounts for a smaller percentage (possibly 10%–12%) of CAE patients and is characterized by the development of tonic-clonic and myoclonic seizures during adolescence, after the onset of absences in childhood.

Pure tonic-clonic epilepsies, including those with seizures on awakening, are reported to account for another 22%–37% of all epilepsies, but, because tonic-clonic seizures, as the sole clinical manifestation of epilepsy, are rare and are preceded by absences in as much as 45% of polygraphically or closed circuit television videotape-EEG recorded attacks, absence epilepsy is more common than reported (Beyer and Jovanovic 1966; Janz 1969; Wolf 1992). Therefore, absence epilepsies, including the


I 201
N-branch
G-branch
D8S557
D8S558
D8S529
D8S256
D8S537
D8S1710
D8S272
D8S554
D8S534
D8S1783
D8S274
D8S502
D8S523
D8S1753
D8S1761
D8S1837

three subsyndromes of CAE mentioned above, may account for as much as 5%–15% of all epilepsies.

A major, if not exclusive, role for genetics in the etiology of CAE was established by twin studies. Lennox and Lennox (1960) confined analyses to EEG spike and wave traits, for 30 pairs of twins. Concordance was 74% in MZ twins and 27% in DZ twins. If twin pairs were examined at the peak age of phenotypic expression (6–18 years), the concordance rate for absence and tonic-clonic epilepsies was 100% for MZ twins.

Metrakos and Metrakos (1961) showed that the EEG trait of a 3-Hz spike and slow-wave complex is inherited as an autosomal dominant gene with maximum penetrance at 10 years of age (range 4.5–16.5 years). The EEG trait disappears rapidly in older age groups and is very seldom present by 40 years of age. Metrakos and Metrakos (1961) also observed that siblings and offspring had a 50% risk of inheriting the EEG trait, a 35% risk of expression of the EEG trait in their lifetime, a 12% risk for generalized tonic-clonic seizures, and an 8% risk for absences. Despite these seminal studies on the clinical genetics of CAE and its typical EEG trait, the chromosome locus for this syndrome has remained unknown.

In this article, we report a large five-generation family from Bombay, India, with a proband and 10 other family members who suffer from clinical CAE and/or the EEG 3–4-Hz spike and multispike–slow wave complexes. Model-free and model-based linkage analyses identified the CAE locus to be on chromosome 8q24. Haplotypes and two recombinations identified a 3.2-cM interval in 8q24. We extended linkage analyses to five smaller multiplex families with the same epilepsy syndrome, and we obtained positive LOD scores, haplotypes that segregated with affecteds, and three recombinations that identified the same 8q24 locus.

Subjects and Methods

Patient and Family Database

One large family from Bombay, India, and five smaller multiplex families were enrolled in this study. Each participating subject or, in the case of minors, the responsible adult signed an informed-consent form, as approved by the Human Subject Protection Committee at the School of Medicine, University of California–Los Angeles (UCLA), or at the participating institution.

A Large Family from Bombay, India

Ascertainment.—We ascertained the large family from Bombay, India (fig. 1), through a 14-year-old proband who fulfilled the inclusion criteria for CAE that are based on the guidelines of the Commission on Classification of the International League against Epilepsy (1989; Loiseau 1992) (see Appendix). The criteria include the following: (1) absences having started sometime during the 3d–12th year of life; (2) 1–20 absences/d and at least 1 absence/d; (3) clinical absence seizures observed during 3–4-Hz spike and multispike–slow wave complexes, after 3 min of hyperventilation, and EEG spike waves that were not photosensitive; (4) Tonic-clonic or clonic-tonic-clonic convulsions in adolescence; and (5) normal physical examination and computerized tomography of the brain.

Clinical and EEG validation of family members.—We performed EEGs and confirmed the electroclinical state of 78 family members (fig. 1). Individuals with a history of seizures were clinically examined by at least one investigator from UCLA and two investigators from K. E. M. Hospital, Bombay. All EEG tracings were read and interpreted by at least two electroencephalographers. After all the clinical and EEG data were gathered and before any genotyping was performed, clinical and EEG affected or unaffected status was assigned to the family members.

Phenotypes

Absences, tonic-clonic seizures, and EEG 3–4-Hz spike and multispike–slow wave complexes.—Of the 78 family members examined, 17 had either seizures or paroxysmal epileptiform discharges in their EEGs (fig. 1). Eleven individuals (24, 64, 66, 72, 112, 113, 114, 115, 117, 172B, and 81) either complained of absences or

Figure 1 Pedigree of family from India, showing segregation of microsatellite markers on chromosome 8q. APM and two-point linkage analyses involved 78 family members, from five generations, who were genotyped; 10 of these members are not represented in this figure, because they were unaffected and uninformative. Squares and circles indicate males and females, respectively. Unblackened symbols indicate unaffected individuals. Blackened symbols indicate individuals affected with absence (24, 66, 81, 115, and 172B), absence plus tonic-clonic seizures (3, 6, 9, 34, 58, 64, 112, 113, and 114), or absence in remission (117). Stippled symbols indicate individuals affected with partial seizures (45 and 69) or Rolandic epilepsy (126). The unblackened symbol with a dot in the middle indicates an individual with febrile convulsions (85). Electroencephalography showed three EEG patterns, namely, 3–4-Hz diffuse spike and multispike–slow wave complexes (24, 64, 66, 72, 81, 112, 113, 114, 115, 117, and 172B); an occipital delta (66); and bifrontal spike waves and short segments of diffuse spike waves (45). Age at onset of absences was 3.5–10 years, except for 72, whose frequent attacks of absences had started at 14 years of age. Onset of absences in 3 was during childhood, but age could not be specified. Ages at onset for 45 and 69 were 19 and 17 years, respectively. For 126, age at onset of seizures was 3 years. Note the recombinations in 172B and 81. The centromeric border is defined by crossovers in 172B and 81, between D8S1710 and D8S272, whereas the telomeric border is shown by crossovers between D8S523 and D8S502.

had 3–4-Hz spike and multispike–slow wave complexes during actual attacks; all these 11 individuals also had interictal 3–5-Hz spike and multispike–slow wave complexes, as well as 3–5-Hz sharp and slow wave complexes. Five of these patients had developed tonic-clonic seizures during late childhood or during adolescence.

Six family members (117, 24, 115, 66, 81, and 172B) had active or clinically undiagnosed absences as the sole phenotype. Only one person complained of frequent daily absences; five family members had their absences determined by their EEGs (fig. 2). Family members 115 (12 years old), 172B (5 years old), 24 (32 years old), 66 (8 years old), and 81 (8 years old) had spontaneous regular and irregular 3–4-Hz spike and multispike–slow wave complexes that lasted 1.5–3 s. These EEG patterns are almost always (98%) associated with clinical absences (Dalby 1969; Blume 1982). Family member 24, who is the mother of family member 81, had 3–4-Hz spike and multispike–slow wave complexes that were activated by photic stimulation. Photic stimulation can precipitate spike and wave bursts in 13% of absence patients (Dalby 1969). Even though these individuals were not complaining of clinical absences, we considered them to be definitely affected with the EEG trait of absence and with undiagnosed clinical absences.

Family members with partial seizures and secondary tonic-clonic convulsions.—Family members 45 and 69 had their first seizures at 19 and 17 years of age, respectively. Both complained of auras or warnings of "electric sensation in the head" and "flashlike sensation in the mind," preceding generalized tonic-clonic seizures with adverse head- and eye-turning to one side. The EEGs of family member 45 showed long bursts of 4–5-Hz generalized spikes, sharp and slow wave formations, and bifrontal spike waves. The EEGs of family member 69 were normal on two occasions.

Family member 126 had Rolandic seizures. Beginning at 3 years of age, she experienced clonic jerks of the right limbs and the facial muscles on one side, accompanied by head- and eye-turning to one side. Most sei-

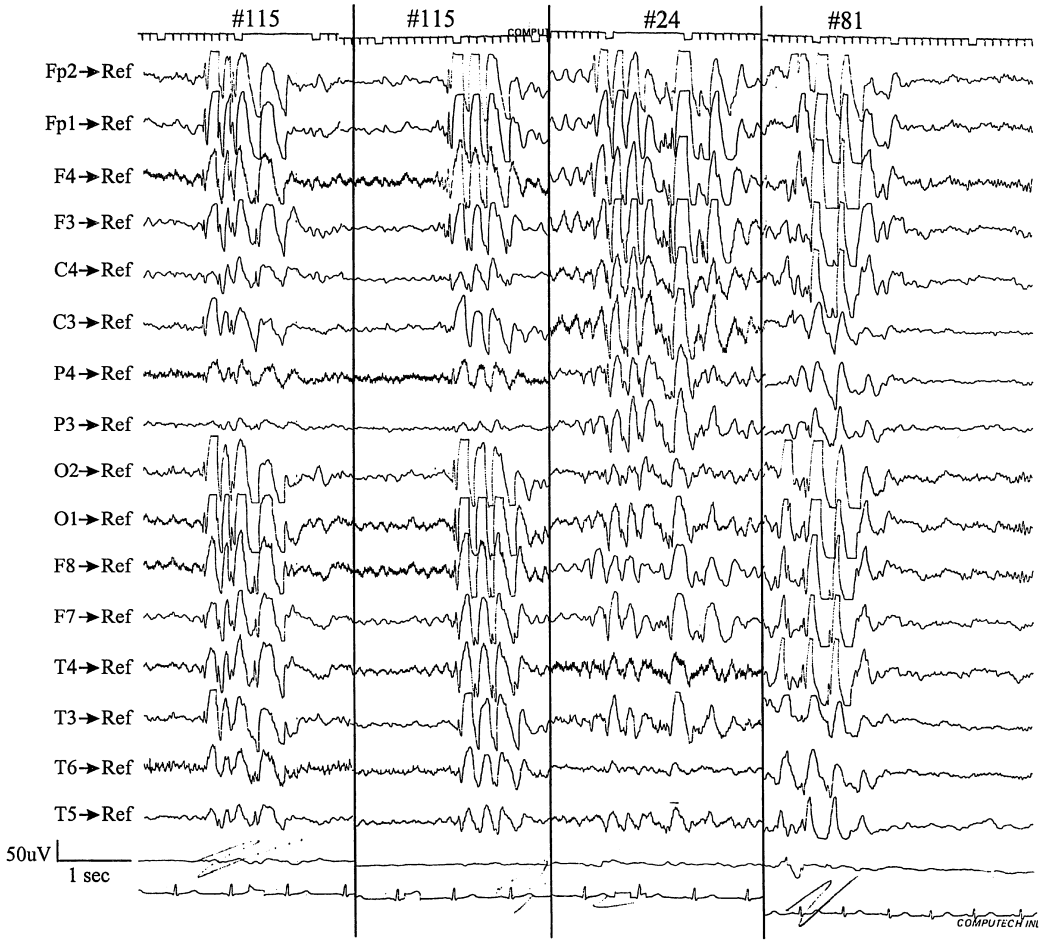


Figure 2 Examples of EEG 3–4-Hz spike and multispike–slow wave complexes in members (115, 24, and 81) of the family from Bombay, India, who were suspected to have undiagnosed clinical absences.

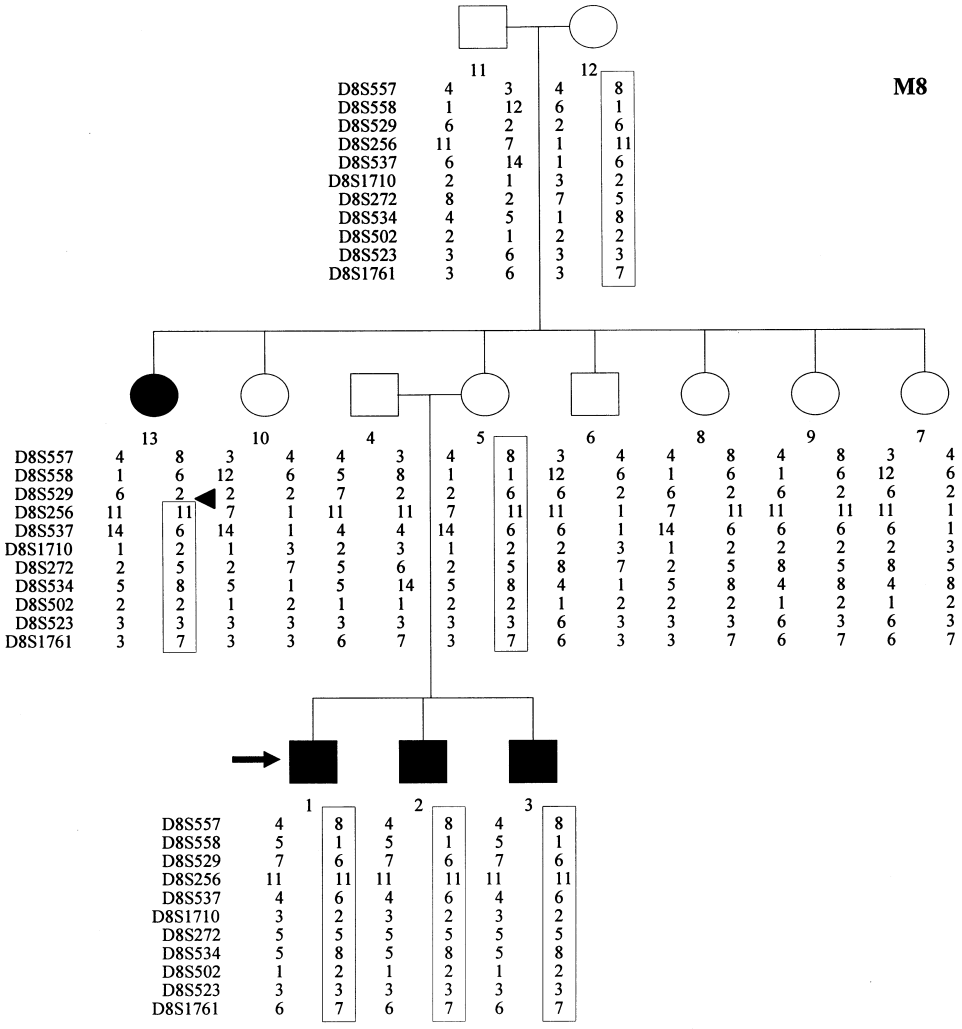


Figure 3 Multiplex CAE family from Spain (M8), showing segregation of chromosome 8q24 microsatellites and three affected brothers and an affected aunt. Note the crossover event between D8S529 and D8S256. Symbols are defined as in fig. 1.

zures occurred at night. EEGs showed paroxysmal spikes and sharp waves over the left centrotemporal areas. Magnetic-resonance imaging and computerized-tomography brain scans were normal.

Family members with infantile febrile convulsions.—Three other family members (85, 146, and 150), who had had febrile convulsions in infancy, were considered to be unaffected, for linkage analyses.

Deceased family members who had had epileptic seizures.—Family member 3 had suffered from lifelong generalized tonic-clonic convulsions, with absences known to have started during childhood. He had drowned in a river during a convulsive seizure, at 35 years of age. Family member 9 also had drowned in a river, at 16 years of age. He had suffered from generalized tonic-clonic convulsions that had started at 5 years of age. Family member 58 had died at 12 years of age, because of convulsive-status epilepticus. Her seizures had started in early childhood.

Five Smaller Multiplex Families

Ascertainment.—Five smaller multiplex families (family 112 from Argentina, family 130 from California, families M8 and M2 from Spain, and family S302 from Saudi Arabia; figs. 3–5) were ascertained through a proband with frequent daily childhood absences and with tonic-clonic seizures persisting into adolescence and adulthood and associated with EEG diffuse 3–4-Hz spike and multispike–slow wave complexes (see Appendix).

Clinical and EEG validation of family members.—Family 112 had three affected female members, all of whom had absences and EEG 3–4-Hz spike and

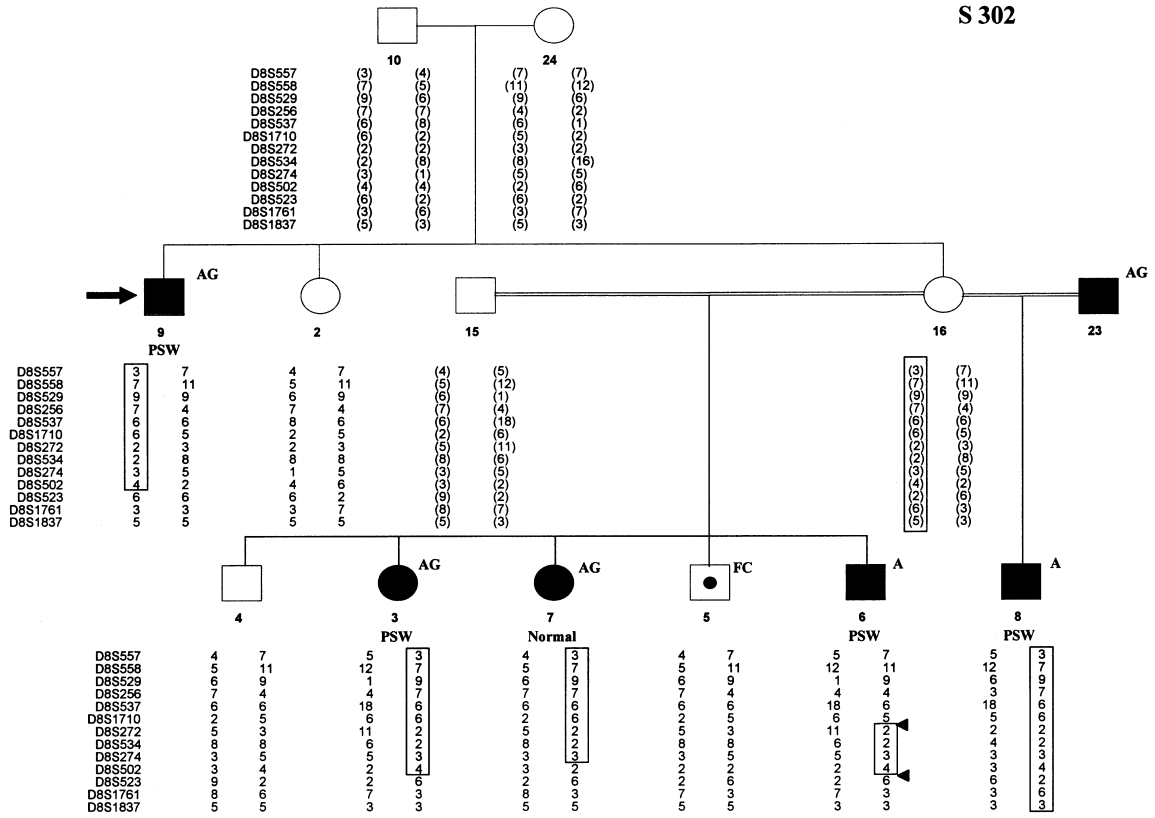


Figure 4 Multiplex CAE family from Saudi Arabia, showing segregation of chromosome 8q24 microsatellite markers and affected members. Note that a recombination is probably present between D8S1710 and D8S272 centromeric and between D8S523 and D8S502 telomeric, as indicated by the arrowheads with family member 6. Symbols are defined as in fig. 1.

multispike–slow wave complexes. The proband had frequent daily absences that had started at 8 years of age and tonic-clonic convulsions that had started at 9 years of age. Two offspring, who are 9 and 16 years of age, started having frequent daily absences at 7 years of age. Another offspring, who is 13 years of age, is unaffected.

Family 130 had two affected brothers, both of whom had absences with 3–4-Hz spike and multispike–slow wave complexes in their EEGs.

Family M8 had three male siblings and a maternal aunt with absences that had started at 3.5–4 years of age and EEG 3–5-Hz spike and multispike–slow wave complexes. The proband is 19 years of age, and his absences have persisted. He has had four episodes of tonic-clonic convulsions. His 12-year-old and 16-year-old brothers both have had rare tonic-clonic seizures and persisting absences.

Family M2 had two half sisters with frequent daily absences that had started at 5 years of age and tonic-clonic seizures that had started at 8 years of age. These two half sisters share a common mother. One half sister is a member of a pair of DZ twins.

Family S302 had a 25-year-old male proband who started having absences (12 attacks/d) at 10 years of age. Occasional tonic-clonic seizures started at 11 years of age, and he had one bout of absence status. Two nieces, 15 and 18 years of age, also suffered from tonic-clonic convulsions. The 18-year-old niece also had absences. One 15-year-old nephew started having absences with myoclonic jerks during attacks, at 11 years of age. A 19-year-old nephew had suffered from one episode of febrile seizures, at 4 years of age, and was considered to be unaffected, for linkage analyses. An 8-year-old, male, first-degree cousin had absences with myoclonic jerks during convulsions.

DNA Analyses and Genotyping

Genomic DNA was extracted from peripheral blood, by means of phenol/chloroform, followed by isopropanol precipitation (Sambrook et al. 1989) or by use of the QUIAamp blood kit (Qiagen). Genotyping was performed on the six families with persisting CAE, by use of the method described by Weber and May (1989). No other families with remitting CAE were genotyped. We chose highly polymorphic short tandem repeats or microsatellites, from Research Genetics, with heterozygosity of >.7. Allelic scoring was performed by at least two

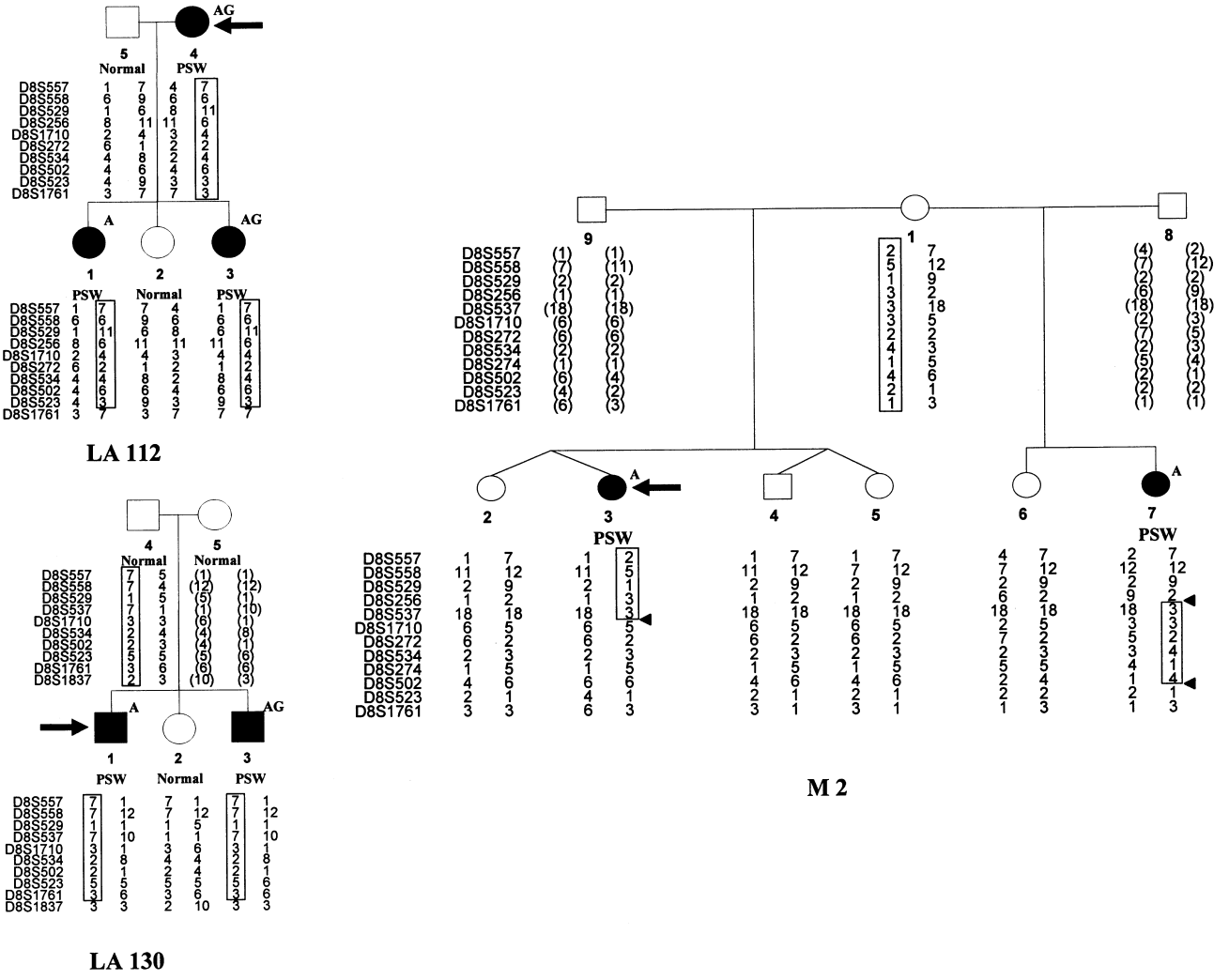


Figure 5 Three small multiplex CAE families, from Argentina (LA112), Los Angeles (LA130), and Madrid, Spain (M2), showing segregation of chromosome 8q24 microsatellites and affected members. Note the recombinations in members 3 and 7 of family M2. The centromeric border is defined by a crossover between D8S1710 and D8S537 in family member 3 and a crossover between D8S537 and D8S256 in family member 7. The telomeric border is shown by the crossover between D8S502 and D8S523 in family member 7. Abbreviations and their meanings are similar to those used in fig. 1.

of the authors (C.Y.F. and Y.H.), who did not know the clinical status of the family members at the time of the scoring.

Loci Studied

Because of previous reports of genetic linkage between (1) classic juvenile myoclonic epilepsy and chromosome 6p (Liu et al. 1995; Serratosa et al. 1996), (2) juvenile myoclonic epilepsy mixed with absences and chromosome 1p (Westling et al. 1996), and (3) idiopathic generalized epilepsies and chromosome 8q (Zara et al. 1995), genomic screening of the family from Bombay was started with microsatellites from chromosomes 6p, 8q, and 1p, by use of the model-free affected–pedigree member (APM) method and the model-based LOD-score method. Initially, we screened the whole human genome, using 169 microsatellites and, eventually, 300 microsatellites (Weber versions 5 and 6) (Dib et al. 1996).

Data for primer sequences, allele sizes, and frequencies of each marker were obtained from the Genome Database. Members of a CEPH reference family were used during the scoring of genotypes. New allele numbers were created on the basis of the new marker sizes discovered during the genotyping process.

Linkage Analyses

Under the assumption of autosomal dominant inheritance with 50% penetrance and a linked codominant marker with eight alleles, SLINK (version 2.60) considered individuals with CAE and/or 3–4-Hz spike and mul-

tispike–slow waves complexes to be affected and predicted an average LOD score (ELOD) of 4.3 at a recombination fraction (θ) of .00, after 2,000 replicates, with an SD of 1.333573 (Ott 1989; Weeks et al. 1990).

For the actual linkage analyses, we tested two diagnostic models. For the narrow diagnostic model, the phenotypes of affected members included absence epilepsy, 3–4-Hz spike and multispike–slow wave complexes, and/or tonic-clonic seizures. The broad diagnostic model included affected members with absences, tonic-clonic convulsions, partial seizures, secondary generalized tonic-clonic convulsions, and Rolandic seizures. Patients whose only phenotype consisted of febrile convulsions in infancy or early childhood were classified as unaffected.

The APM method (Weeks and Lange 1988) was used to screen for microsatellites that showed potential linkage during the initial screens of chromosomes 1p, 6p, and 8q, as well as during the subsequent genomewide screen. Parametric, or model-dependent, two-point linkage analyses then were performed by use of the computer program MLINK in the LINKAGE software package, version 5.1 (Ott 1974). The marker allele frequencies were obtained from CEPH and from the "married-in" family members. The frequency of the disease allele was estimated to be .001. Phenocopy and gene-mutation rates were set as 0. An autosomal dominant mode of transmission was used for the model-dependent linkage analyses. An age-at-onset penetrance curve derived from 114 CAE patients was used to assign each family member to a liability class. In these four liability classes, we assumed (1) a penetrance of 5% at 0–3 years of age; (2) a penetrance of 80% at 3–14 years of age; (3) a penetrance of 40% at 14–30 years of age; and (4) a penetrance of 10% at $\geqslant$31 years of age. LOD scores were calculated at a sex θ, male equal to female ($\theta_{[m=f]}$), over a grid of θ values of .00, .05, .10, .15, .20, .25, .30, .35, and .40.

Haplotype Analysis

We constructed haplotypes by using the computer program Cyrillic (Cherwell Scientific), version 2.0. Sixteen markers located on chromosome 8q were used (figs. 1 and 6). We followed the 1996 Généthon genetic map and consulted the map positions and order of microsatellites on the YAC contig map from Généthon (Chumakov et al. 1995) and the radiation-hybrid maps of the Whitehead Institute for Biomedical Research/MIT Center for Genome Research and of the Stanford Human Genome Center.

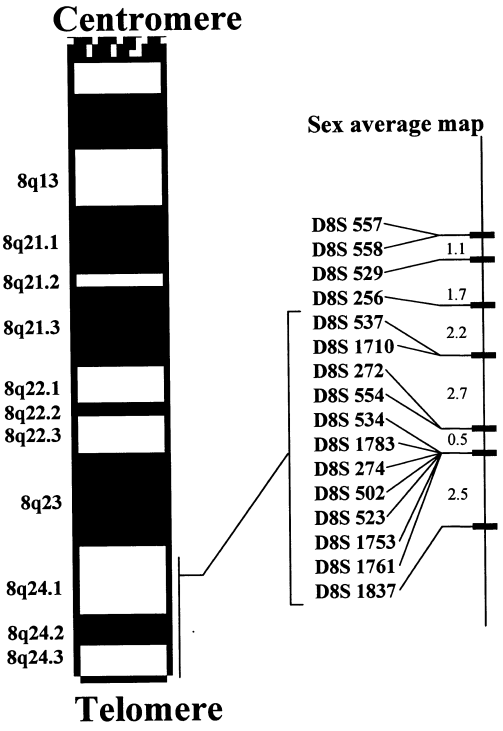


Figure 6 Chromosome 8q24 backbone. Polymorphic markers used for linkage analyses and haplotype construction are represented. The CAE gene has been mapped between D8S1710 and D8S502.

Results

Model-Free Linkage Analyses of the Family from Bombay, India

The large family from Bombay, India, was used as the pilot family, to search for a chromosomal locus for CAE and its EEG trait. Eleven affected members had absence and/or 3–4-Hz spike and multispike–slow wave complexes in their EEGs. During the initial screening with chromosomes 6p, 8q, and 1p markers, the APM method utilized the narrow diagnostic model. The APM method showed significant P values [$f(P) = 1/\sqrt{P}$] of .00000–.02 for nine markers on chromosome 8q24, suggesting linkage of the affected members to markers D8S256, D8S537, D8S534, and D8S1753 (P = .00000), D8S274 (P = .00014), D8S1783 (P = .00012), D8S502 (P = .00199), D8S272 (P = .00624), and D8S1761 (P = .02875). In our analyses, we first utilized the allele frequencies reported for each marker in the Genome Database. Our second set of analyses utilized the allele frequencies of the married-in members of the large family from India, and the robustness of the results did not diminish.

Model-Based Linkage Analyses of the Family from Bombay, India

Segregation of the absence phenotype followed an autosomal dominant mode with incomplete penetrance. Prior to simulation and actual linkage analyses, we calculated the number of family members with absence epilepsy and/or EEG spike waves, across four sibships in which the epilepsy trait appeared to be present. From matings of family members 36 and 37, 38 and 39, 20 and 21, and 18 and 19, we estimated the penetrance to be 50% (fig. 1). After linkage to chromosome 8q24 markers was confirmed, we reevaluated the penetrance of the gene, using haplotype analyses. Eleven of 21 family members showing the haplotype segregating with the disease were clinically affected with absences, indicating a penetrance of almost 50%.

After significant results were obtained by use of the APM method, we used more markers in the chromosome 8q region (D8S557, D8S558, D8S529, D8S1710, D8S537, D8S554, D8S1753, D8S1783, D8S1761, D8S523, and D8S1837). For the two-point linkage analysis, we assumed an autosomal dominant mode of inheritance, assigned family members to liability classes, and used the narrow diagnostic model. The resulting LOD scores, across different θ values, are shown in table 1. A maximum LOD score (Z_{max}) of 3.58, with $\theta_{(m=f)} = .00$, was found for D8S502. D8S272, which is located ~0.5 cM away from D8S502, also gave a positive but not significant LOD score of 1.135, with $\theta_{(m=f)} = .00$. When we used the broader diagnostic model, which classified individuals with partial seizures (126, 45, and 69) as affected, to perform the two-point linkage analysis, the LOD score of marker D8S502 dropped from 3.6 to 1.1, with $\theta_{(m=f)} = .00$. No informative recombination events were present in family members affected with partial seizures.

On the first pass, the human-genome screen revealed five chromosomal regions with positive LOD scores of 0.8–1.2 (D1S207, D6S282, D10S1426, D11S1986, and D15S144). Multiple microsatellites then were used to "flood" the areas surrounding these microsatellites on chromosomes 1p, 6p, 10p, 11q, and 15p. None of the microsatellites reached significant LOD scores. The highest LOD score was 1.8, at $\theta = .00$, for D1S436 located at the telomeric end of chromosome 1p. We constructed haplotypes composed of 15 markers, from D1S507 to D1S233, but the alleles did not segregate with affected members.

Haplotype Analysis and Recombination Mapping of Chromosome 8q of the Family from Bombay, India

We presumed that family members 7, 36, 112, 110, and 32 exhibited the ancestral haplotype on 8q24, spanning D8S557–D8S1837. A smaller segment of this ancestral haplotype, comprising identical alleles spanned by markers D8S272 and D8S274, was present in all individuals with CAE and/or 3–4-Hz spike and multispike–slow wave complexes (fig. 1). This segment also was present in some asymptomatic carriers. Family members 114, 72, 172B, 66, 24, and 81 showed haplotypes with a centromeric border of D8S1710 flanked by D8S537. Two recombinations in family members 172B and 81 confirmed a centromeric border of D8S1710 flanked by D8S537. However, family members 117 and 115 reduced the size of the CAE region further by identification of D8S272 as a centromeric end flanked by D8S1710. Six affected members (72, 64, 172B, 66, 24, and 81) identified a telomeric border of D8S502 flanked by D8S523. These haplotypes positioned the CAE gene in a 3.2-cM interval flanked by D8S1710 centromeric and D8S523 telomeric (see fig. 6 for the chromosome 8q24 microsatellite map).

Several individuals (36, 38, and 32, who are offspring of individuals 8 and 7) in the N branch of the family have extended regions of homozygous markers, from D8S272/D8S554 to D8S1761/D8S1837 (fig. 1). All these members were classified as unaffected, because their clinical and EEG examinations did not disclose epilepsy. Note that family member 114 had inherited her epilepsy haplotype from the chromosome of family member 38

Table 1

LOD Scores and θ Values for the Family from Bombay, India

Microsatellite Marker	LOD Score at θ =								
	.00	.05	.10	.15	.20	.25	.30	.35	.40
D8S272	1.196	1.006	.853	.726	.613	.500	.381	.255	.132
D8S554	.645	.588	.524	.454	.305	.230	.158	.095	.400
D8S534	.328	.277	.227	.180	.136	.096	.063	.036	.017
D8S1783	.047	.127	.160	.162	.143	.113	.078	.044	.018
D8S274	.095	.083	.079	.076	.072	.063	.052	.039	.025
D8S502	3.588	3.251	2.901	2.539	2.167	1.783	1.388	.987	.593
D8S523	−.145	1.745	1.882	1.834	1.687	1.470	1.198	.883	.542
D8S1753	−2.569	−.849	−.545	−.377	−.265	−.183	−.119	−.069	−.032
D8S1761	.126	.107	.100	.094	.086	.074	.058	.041	.024

that had originated from the grandmother (family member 8). All other affected offspring (117, 115, and 113) of family member 38 had inherited their epilepsy haplotypes from the other chromosome of family member 38, which was passed on by their grandfather (family member 7). However, this epilepsy haplotype from family member 7 had originated from one chromosome from the great-grandmother (family member 2).

In the G branch of the family, the fourth-generation members with CAE (64, 172B, 66, 24, and 81) had inherited their epilepsy haplotypes from family members 18 and 24. This epilepsy haplotype had been passed on, in turn, by family member 5, who is of the second generation, and originally had been contained in one chromosome of their great-grandfather (family member 3). Only after haplotypes were constructed did we realize that family members 85 (affected with febrile convulsions and multispike–slow wave complexes), 45 (affected with partial seizures and secondary tonic-clonic convulsions with bifrontal spike waves), and 126 (affected with Rolandic seizures) also carried the epilepsy haplotype that had been transmitted to them originally by their great-grandmother (family member 2). Since the great-grandmother carried the same 8q24 epilepsy haplotype and had passed this epilepsy haplotype to family members 85, 45, and 126, the syndromes of febrile convulsions, tonic-clonic seizures with auras, and Rolandic seizures could be part of the phenotypes resulting from the 8q24 epilepsy gene. If this is true, family members 85 and 45 position the CAE gene in a 0.5-cM area flanked by D8S272 and D8S502.

Extension to Five Smaller Multiplex Families

In the five smaller multiplex families, there were 15 affected members (including the probands) (figs. 3–5). Thirteen had frequent daily absences that had started during childhood, 1 nonproband member had frequent daily absences that had started during adolescence, and 1 nonproband individual had tonic-clonic convulsions only. Of the 13 members with childhood absences, 5 also had tonic-clonic seizures. As had been done for the large family from Bombay, ascertainment was through a proband with persisting CAE; clinical and EEG states of affectedness were assigned to family members prior to genotyping. We then analyzed the data by first using the APM method, and we obtained significant results, using the $f(P) = 1/\sqrt{P}$ statistic, with 10 microsatellites—namely, D8S557 ($P = .00004$), D8S558, D8S529, D8S256, D8S537, D8S534, D8S1761, and D8S523 ($P = .00000$), D8S502 ($P = .08822$), and D8S272 ($P = .00881$).

We also obtained positive pooled LOD scores or Z_{max} scores during model-dependent linkage analyses, for DS537 (2.4 at $\theta = .00$) and D8S1761 (1.7 at $\theta = .00$). More importantly, haplotypes comprising the same 8q24 microsatellites segregated with affected members in each of these five families, which were from various countries on different continents (figs. 3–5). In addition, recombinations in the two small multiplex families from Spain and Saudi Arabia were informative for a locus in the same 8q24 area that was critical for the large family from India. Families 130 from Los Angeles and LA112 from Argentina (fig. 5) and M8 from Spain (fig. 3) did not have any informative recombinations in affected members.

Haplotypes in family members 9, 3, 7, and 8 of family S302 from Saudi Arabia (fig. 4) defined an interval occupied by D8S557 centromeric and D8S502–D8S1837 telomeric. The telomeric border was cut to D8S274 flanked by D8S502, by family members 3 and 7. Family member 6 cut the centromeric border to D8S272 flanked by D8S1710. This positions the CAE gene in the same 3.2-cM region flanked by D8S1710 and D8S502, found in the large family from India.

Recombinations in family members 3 and 7 of family M2 from Spain may be informative. Crossovers between D8S537 and D8S1710, at the telomeric end, are present in family member 3, suggesting that the CAE gene is in a small area, of <1 cM, between D8S537 and D8S1710 (fig. 5). D8S1710 is still within the D8S1710–D8S502 vicinity identified for CAE by the large family from India. However, if the important marker is D8S537, then family M2 from Spain did not show linkage to the same region to which the family from India showed linkage. In family member 7, crossovers between D8S256 and D8S537 (centromeric) and between D8S502 and D8S523 (telomeric) are present (fig. 5). These crossovers in family member 7 suggest a 5.4-cM critical region between D8S256 and D8S523. The D8S1710–D8S502 area identified for CAE by the large family from India is within the region spanned by D8S256 and D8S523. Thus, after correlating the crossovers in family members 3 (which included D8S1710) and 7 (which defined an area spanned by D8S256 and D8S523 and which included D8S1710–D8S502), we favor the suggestion that this family showed linkage to the same area to which the family from India showed linkage.

Discussion

Previous studies of the genetics of idiopathic generalized epilepsies have shown that different epilepsy syndromes commonly occur in members of the same family (Delgado-Escueta et al. 1990; Durner et al. 1991; Whitehouse et al. 1993). This causes problems when considering who should be labeled as affected, for linkage analysis. In the large pedigree from Bombay, India, we were able to use a narrow diagnostic model for linkage analyses, because there were enough persons affected with

the same persisting CAE phenotype. There was only one family member whose frequent daily absences could be traced back to only 14 years of age. He does not have juvenile absences, because absences are rare in such syndromes and usually are preceded by tonic-clonic seizures (Commission on Classification of the International League against Epilepsy 1989). In this family member (72), frequent daily absences preceded the appearance of tonic-clonic seizures at 19 years of age. For these reasons and because he had the EEG hallmark of CAE, we considered him to be affected. The narrow diagnostic model considered as affected only persons with CAE and its characteristic EEG trait of 3–4-Hz spike and multispike–slow wave complexes. In so doing, we had hoped to study a homogenous epilepsy phenotype that was vertically transmitted and Mendelian in inheritance. In the more moderate-sized families, we encountered one member with tonic-clonic seizures as the sole phenotype and two members with childhood absences with myoclonic jerks during attacks. Because mild myoclonic jerks can occur during absence attacks in CAE (Loiseau 1992) and because they all had the EEG trait of 3–4-Hz spike and multispike–slow wave complexes, we considered these members to be affected.

The EEG not only complemented the clinical diagnosis of CAE in actively symptomatic members but also revealed the presence of undiagnosed absences and 3–4-Hz spike and multispike–slow wave complexes in four asymptomatic children and in one asymptomatic adult, in the large family from India. Without EEGs, these five persons would have been considered to be unaffected, for linkage analyses. With misdiagnosis and misclassification of epilepsy-affected persons as unaffected, real linkage may be overlooked and significantly more data would be needed in order to obtain the same power to detect linkage (Ott 1991).

Analysis, by SLINK, of the family from India produced an ELOD of 4.3; the observed LOD score of 3.58 for D8S502 (at $\theta = .00$) is within 1 SD of the ELOD. The Z_{max} was 3.58 for D8S502 (at $\theta = .00$), under the model of autosomal dominant inheritance with 50% penetrance and four age-dependent liability classes. Two other markers provided LOD scores of 1.2 (for D8S272 at $\theta = .00$) and 1.9 (for D8S523 at $\theta = .00$). In classic model-based linkage, a LOD score >3 usually is required in order to provide a prima facie case for linkage and for the presence of a major-gene locus (Morton 1955). According to Morton (1998), "with rare exceptions, linkage at a correctly computed LOD >3, is true" (p. 692). Morton also argues that multiple markers and candidates have not invalidated the canonical LOD-3 criterion for major loci. If we wish to account for both multiple-marker tests, as suggested by Lander and Kruglyak (1995), and for corrections for multiple models tested, as suggested by Risch (1991), the final threshold for significant linkage would be 3.5. With a LOD score of 3.58, D8S502 still would provide significant evidence for linkage.

For five smaller multiplex families—each ascertained through a proband with the same persisting CAE syndrome and recruited as independent samples, from Spain, Saudi Arabia, Argentina, and Los Angeles—we obtained positive LOD scores (Z_{max} of 2.07 for D8S537) and segregating chromosome 8q24 haplotypes. These results suggest that the epilepsy in these families is genetically the same as the persisting CAE of the family from India, which showed linkage to chromosome 8q24. Chromosome 8q24 haplotypes segregated with affected members, and meiotic recombinations in two of these smaller families defined the same critical CAE region previously observed in the large family from India, namely, the 3.2-cM CAE interval flanked by D8S1710 and D8S502.

Zara et al. (1995) studied five families with CAE, two families with juvenile myoclonic epilepsy, one family with tonic-clonic epilepsy, and two families with febrile seizures. In four of these families, different idiopathic generalized epilepsy syndromes occurred in members of the same family. Despite these mixtures of disparate epilepsy syndromes, highly significant results were obtained with marker D8S256, by use of the extended–sib pair and APM methods. However, analyses by a model-dependent method did not obtain significant LOD scores. The highest LOD score (1.96 at $\theta_{[m=f]} = .05$) was obtained by use of an autosomal recessive model with 90% penetrance. Nonetheless, these authors contended that a susceptibility gene for idiopathic generalized epilepsies was very likely to be on chromosome 8q. In 1996, Kruglyak et al. (1996) reanalyzed the data of Zara et al. (1995), using the NPL-statistic sib pairs, and disputed their conclusions. When NPL of GENEHUNTER was applied, the same data from Italy yielded a score of 2.26 at D8S256 ($P = .02$). Complete multipoint analyses involving D8S284, D8S256, and D8S534 yielded a lower score, 1.79 ($P = .63$). Thus, this nonparametric method, which detects allele sharing among affected individuals that are identical by descent, detected no evidence for linkage to chromosome 8q.

Our study could be interpreted as confirmation of the report by Zara et al. (1995). However, our CAE locus on 8q24 is 2.2–4.9 cM telomeric from the D8256 region reported by Zara et al. (1995). Our CAE locus is also 2.2–4.9 cM telomeric to the area for the second genotype of benign familial neonatal convulsions. In this latter epilepsy syndrome, a missense mutation in a voltage-gated K-channel gene (KCNQ3) belonging to the KQT-like subfamily has been found recently. This KCNQ3 gene, however, is outside the critical CAE region. Other, yet to be cloned potassium channels (Chandy and Gutman 1993) could be located in this same CAE 8q24 area

and would be potential candidates for the CAE gene. Other possible candidates for the CAE gene, on 8q24, are (1) the ionotropic NMDA-associated glutamate receptor 1 (Lewis et al. 1996) and (2) the human jerky gene. These latter two genes are located within the critical CAE region, and each has been reported to have a role in experimental epilepsies (Peet et al. 1987; Toth et al. 1995).

In conclusion, we report the first large family that both expresses the phenotype of persisting CAE and independently proves linkage to chromosome 8q24. Extension of our linkage-mapping and haplotype studies to five smaller multiplex families with the same persisting CAE subsyndrome supported linkage to the same 8q24 region. Quick independent replication by use of more new families with persisting CAE and extension to families with remitting CAE are highly desirable at this time.

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Appendix

Inclusion Criteria for Probands with CAE

The following criteria are based on those from the Commission on Classification of the International League against Epilepsy (1989) and from the report by Loiseau (1992).

1. Absence seizures appear as the initial type of attacks, at 3–12 years of age.

2. Absences appear very frequently throughout the day and are spontaneous or precipitated by environmental factors.

3. The EEG during absences shows bilateral synchronous and symmetrical spike-wave discharges; in most patients, spike waves are regular at 3 Hz but may be less regular as 3–5-Hz multispike waves.

4. EEG background activity is normal.

5. Absence seizures may remit spontaneously, but 40% of patients develop generalized tonic-clonic seizures during adolescence or later in life; very few patients have only absence seizures as an adult.

6. General physical and neurological examinations are normal.

Electronic-Database Information

URLs for data in this article are as follows:

Genome Database, http://www.gdb.org/ (for primer sequences, allele sizes, and marker frequencies used in the linkage analyses)

Stanford Human Genome Center, http://shgc-www.stanford.edu/ (for radiation-hybrid maps)

Whitehead Institute for Biomedical Research/MIT Center for Genome Research, http://www-genome.wi.mit.edu/ (for radiation-hybrid maps)

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