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Juvenile Myoclonic Epilepsy Locus in Chromosome 6p21.2-p11: Linkage to Convulsions and Electroencephalography Trait

A. W. Liu,[1,2] A. V. Delgado-Escueta,[1,2,4] J. M. Serratosa[1,2] M. E. Alonso,[5], M. T. Medina,[1,6] M. N. Gee,[1,4] S. Cordova,[5] H. Z. Zhao,[1,2,4] J. M. Spellman,[1,2,4] J. R. Ramos Peek,[5] F. Rubio Donnadieu,[5] and R. S. Sparkes[1,3]

[1]California Comprehensive Epilepsy Program, Departments of [2]Neurology and [3]Medicine, University of California, and [4]Veterans Affairs Southwest Regional Epilepsy Center, Neurology and Research Services, West Los Angeles Department of Veterans Affairs Medical Center Los Angeles, Los Angeles; [5]National Institute of Neurology and Neurosurgery, Mexico City; and [6]National Autonomous University, Tegucigalpa, Honduras

Summary

Despite affecting 4 million Americans and 100–200 million persons worldwide, the precise molecular mechanisms of human epilepsies remain unknown. Juvenile myoclonic epilepsy (JME) is the most frequent and, hence, most important form of hereditary grand mal epilepsy. In this epilepsy, electroencephalographic (EEG) 15–30-Hz multispikes produce myoclonic and tonic-clonic convulsions beginning at 8–20 years of age. Moreover, EEG 3.5–6-Hz multispike wave complexes appear in clinically asymptomatic family members. We first studied 38 members of a four-generation LA-Belize family with classical JME but with no pyknoleptic absences. Five living members had JME; four clinically asymptomatic members had EEG multispike wave complexes. Pairwise analysis tightly linked microsatellites centromeric to HLA, namely D6S272 (peak lod score [Z_{max}] = 3.564–3.560 at male-female recombination [$\theta_{m=f}$] = 0–.001) and D6S257 (Z_{max} = 3.672–3.6667 at $\theta_{m=f}$ = 0–.001), spanning 7 cM, to convulsive seizures and EEG multispike wave complexes. A recombination between D6S276 and D6S273 in one affected member placed the JME locus within or below HLA. Pairwise, multipoint, and recombination analyses in this large family independently proved that a JME gene is located in chromosome 6p, centromeric to HLA. We next screened, with the same chromosome 6p21.2-p11 short tandem-repeat polymorphic markers, seven multiplex pedigrees with classic JME. When lod scores for small multiplex families are added to lod scores of the LA-Belize pedigree, Z_{max} values for D6S294 and D6S257 are >7 ($\theta_{m=f}$ = .000). Our results prove that in chromosome 6p21.2-p11 an epilepsy locus exists whose phenotype consists of classic JME with convulsions and/or EEG rapid multispike wave complexes.

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Address for correspondence and reprints: Dr. Antonio V. Delgado-Escueta, Molecular Neuroscience and Neurogenetics Laboratories, Comprehensive Epilepsy Program (W127B), West Los Angeles DVA Medical Center, 11301 Wilshire Boulevard, Los Angeles, CA 90073.

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Introduction

Despite afflicting at least 4 million Americans and 100–200 million persons worldwide (Hauser and Hesdorffer 1993), the precise molecular mechanisms of human epilepsies remain unknown. Interestingly, since 400 B.C. the epilepsies have been suspected to have a hereditary basis: the Hippocratic collection of medical writings argued against superstitions and magicians and declared that "epilepsy is not more divine than other diseases are. Like all diseases, it is hereditary" (Temkin 1971, p. 4). We have been studying juvenile myoclonic epilepsy (JME), the most frequent cause and, hence, most important form of hereditary grand mal (GM) epilepsies. In this epilepsy, rapid 15–30-Hz multispikes and slow wave complexes in the electroencephalograph (EEG) produce myoclonic and tonic-clonic convulsions beginning at 8–20 years of age (Delgado-Escueta and Enrile-Bacsal 1984).

In 1988, we suggested that a gene on chromosome 6p could determine one form of JME (Greenberg et al. 1988*b*). We reported that JME may be linked to properdin factor (Bf) and human leukocyte antigen (HLA) in chromosome 6p. In 11 small JME families, 8 of which are informative for Bf and 3 of which are informative for HLA, lod scores summed to 3.04 (recombination fraction for males [θ_m] = .01; recombination fraction for females [θ_f] = .10), under the assumption of autosomal recessive inheritance with full penetrance. Because we found no significant association with any specific alleles of HLA, and because of one recombinant family, we suggested that the locus may lie outside the HLA region (Greenberg et al. 1988*b*). In 1991, Weissbecker et al. confirmed linkage to HLA serological markers and, by excluding tight linkage to HLA, agreed with our suggestions that the epilepsy locus may lie outside the HLA region (Weissbecker et al. 1991). They reported summed lod scores of 3.11 (θ_m = .001; and θ_f = .20), assuming autosomal dominant inheritance, in 23 small families from Berlin. Using the same Berlin families, Durner et al. (1991) again confirmed linkage but disagreed with our suggestions that the locus could be outside the HLA

region. They reported tight linkage to DNA markers in the HLA-DQ locus, assuming autosomal dominant inheritance with peak lod scores (Z_{max}) of 3.9 at male-female recombination fractions ($\theta_{m=f}$) = .01), when all forms of idiopathic generalized epilepsies were considered affected. Most recently, a new study using 25 small JME families from the United Kingdom and Sweden (Whitehouse et al. 1993) did not segregate a disease gene linked to HLA or other chromosome 6p markers, and genetic heterogeneity was suspected as one possible explanation for this conflicting result.

In the present communication, we first diminished the role of interfamilial genetic heterogeneity, by studying 38 members of a four-generation JME kindred from Los Angeles and Belize (LA-Belize family) that had autosomal dominant inheritance and 70% penetrance. We performed a genomewide search with 146 short tandem-repeat polymorphic markers (STRPs) and found linkage between chromosome 6p21.2-p11 markers and the convulsions and EEG traits of JME. We then screened with the same chromosome 6p21.2-p11 STRPs seven small multiplex pedigrees whose phenotype is similar to that of the LA-Belize family, namely classic JME without pyknoleptic absences (PAs) and 3-Hz spike wave complexes. We also screened with the same chromosome 6p STRPs nine small multiplex JME pedigrees that were atypical for having, in addition, PAs and 3-Hz spike wave complexes.

Subjects, Material, and Methods

1. Classic JME in a Large LA-Belize Family

Clinical and EEG confirmation of phenotypes.—We validated the clinical neurological status (Delgado-Escueta and Enrile-Bacsal 1984) and EEGs (Jasper 1958; Delgado-Escueta et al. 1982) of 11 nuclear and 27 nonnuclear family members who reside in Los Angeles, Corozal (Belize), and Belize City (Belize) (fig. 1). The study was approved by the Human Subjects Protection Committees at the UCLA School of Medicine and the West Los Angeles DVA Medical Center, and informed consent was obtained from all participants. The proband is a 51-year-old male who, like his father (member I-2) and his uncle (member I-3), suffered myoclonic and tonic-clonic convulsions. Epileptic attacks started in the proband at age 10 years. Epileptic seizures also started at age 10 years in the proband's father (member I-2) and the proband's uncle (member I-3). Family members I-2 and I-3 are both deceased and are reliably reported and witnessed by family members to have had JME. We verified myoclonic seizures (MS) on closed-circuit–television videotape and EEG biotelemetry (CCTV-EEG) in the proband. Petit mal absences were not present on CCTV-EEG. Aside from the proband, five other living family members (two male and three female) were also affected with JME. Another living family member (II-5) was affected with an epilepsy syndrome characterized by transient, afebrile neonatal convulsions at age 3 d–6 mo and by febrile seizures at age 3–5 years. Her present EEG shows nonspecific but epileptiform irregular diffuse spike and sharp wave formations. Because she has a form of clinical epilepsy and an epileptiform EEG trait, and because we were unsure whether her clinical syndrome is a form of JME, we classified her as "unknown" for linkage analyses. In the beginning of this report there were four clinically asymptomatic females who showed spontaneous interictal epileptogenic diffuse 3.5–6-Hz multispike and slow wave complexes and bursts of diffuse rapid 4–7-Hz spike and sharp wave formations in their EEGs (see fig. 2). They were classified as affected with the EEG trait of JME. More recently, member III-10 has had rare MS and has suffered from a GM tonic-clonic seizure after gynecologic surgery. General medical and neurological examinations were normal in all members whom we validated.

Genetic parameters and computer simulation.—Results of clinical and EEG examinations of family members determined which components of the diagnostic models could be used for linkage analyses. In a previous study (Greenberg et al. 1988*b*), we used a broad diagnostic model, where members symptomatic with JME and members clinically asymptomatic but with EEG patterns of epileptogenic diffuse 3.5–6-Hz multispike and slow wave complexes and epileptiform bursts of diffuse spike and sharp wave formations were classified as affected. EEG 3.5–6-Hz diffuse polyspike wave complexes are the specific electrophysiological correlates of and produce clinical MS but are present in 0.009% of normal subjects (Zivin and Ajmone-Marsan 1968; Eeg-Olofsson et al. 1971; Cavazzuti et al. 1980) and in 6% of clinically unaffected nuclear relatives of JME patients. Such 3.5–6-Hz polyspike wave paroxysms (see fig. 2) are the hallmark of the EEG trait in asymptomatic members of the LA-Belize family, while JME is the main and predominant phenotype of clinically affected members. Thus, for linkage analyses, we used a broad diagnostic model where family members actively suffering from JME and family members who are clinically asymptomatic but who have the EEG trait are classified as affected.

We classified four family members (II-5, II-8, IV-3, and IV-1) as "unknown" for linkage analyses. Member II-8 refused to have an EEG. We classified IV-3 and IV-1 as "unknown" for linkage analyses, because they are presently <8 years of age. In our analysis, family members <8 years of age (the lowest age for onset of JME) whose EEGs are normal are considered unknown for linkage analysis. An abnormal EEG with 3.5–6-Hz multispike wave complexes would have placed IV-1 and IV-3 in the affected category, under the broad diagnostic model. Persons age 8–20 years

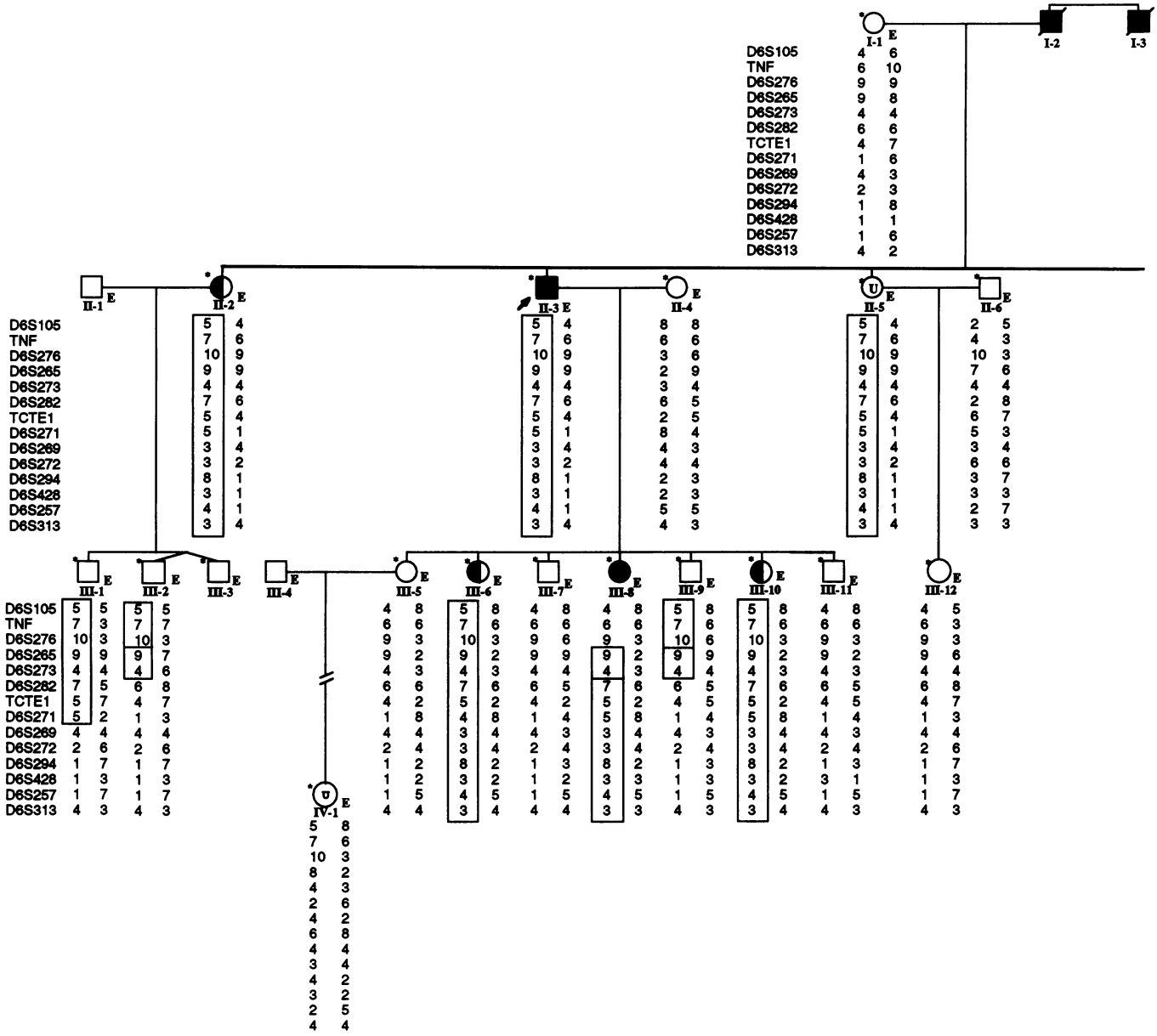
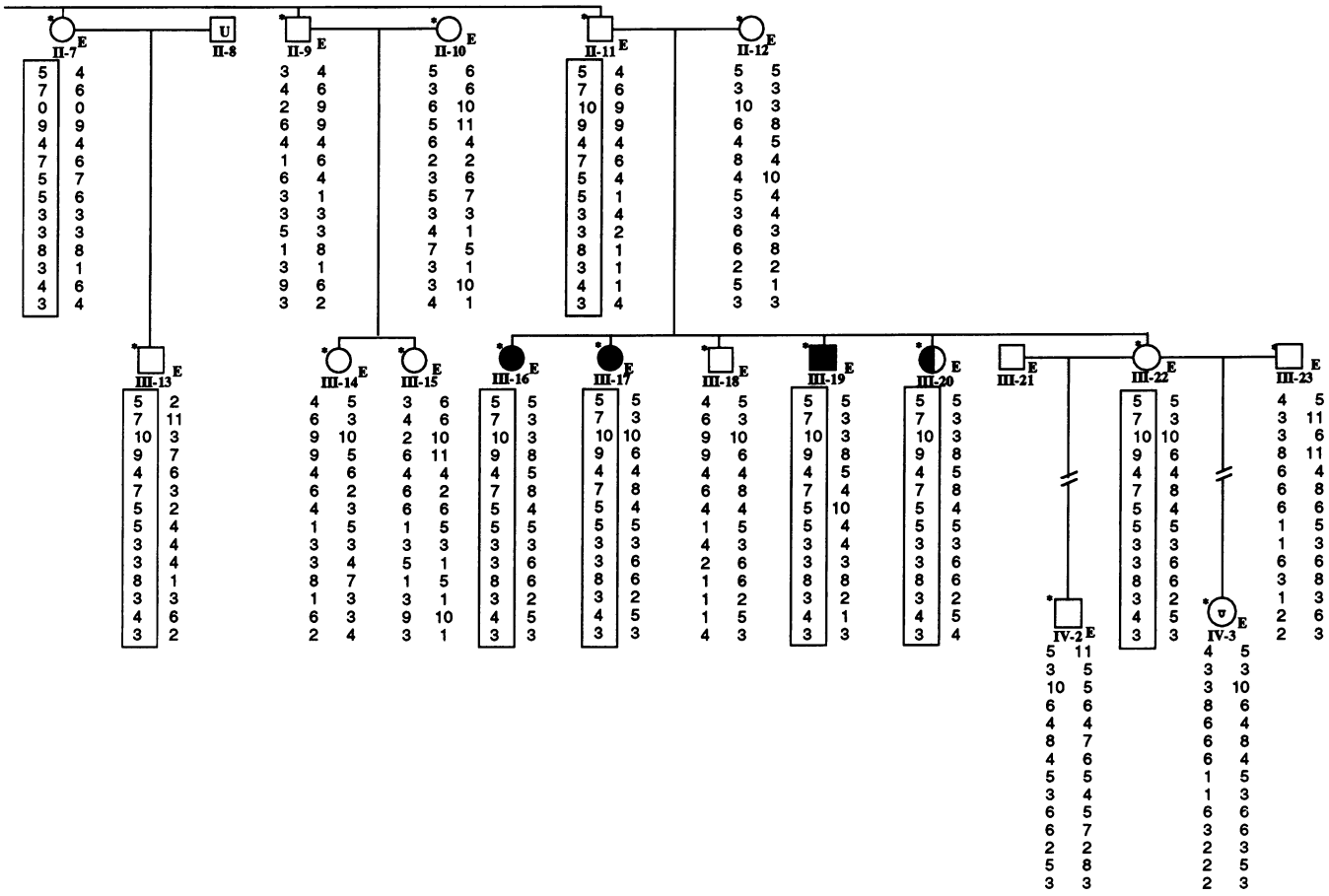


Figure 1 Genetic analysis of a JME family from Los Angeles and Belize. Seven individuals who are clinically affected with JME are represented by fully blackened symbols: II-3 (proband; age at onset, 10 years), I-2 (deceased father of proband; age at onset, 9 years), I-3 (deceased uncle of proband; age at onset, 10 years), III-8 (daughter; age at onset, 13 years), III-16 (niece; age at onset, 13 years), III-17 (niece; age at onset, 8 years), and III-19 (nephew; age at onset, 13 years). The clinical diagnoses of members I-2 and I-3 were based on their histories, obtained from family members; four asymptomatic females diagnosed with EEG 3.5–6-Hz multispike wave complexes are represented by half-blackened symbols; and patients who have normal clinical and EEG examination results are represented by unblackened symbols. An "E" denotes that an individual had an EEG performed; and a "U" is used to denote family members (II-5, II-8, IV-1, and IV-3) who were considered unknown for linkage analysis (see text) testing with the broad diagnostic model. Member II-5 had transient neonatal myoclonic convulsions. IV-1 and IV-3 are <8 years of age. Member II-8 did not have an EEG. Haplotypes have been boxed to aid visual analyses for the following: (1) clinically or EEG affected members; (2) unaffected members who could have informative recombinations; (3) members, such as II-5, whose clinical status is unknown; and (4) clinically and EEG-unaffected members whose haplotypes are similar to those of affected members.

who have no clinical JME and have normal EEGs are considered unaffected.

After clinical and EEG validation of family members determined which components of the broad diagnostic model can be used for linkage analyses, we calculated the proportion of offspring affected with either JME or the EEG polyspike wave complexes (Greenberg et al. 1988*a*; Delgado-Escueta et al. 1994). The proportion of



affected siblings across mating types was .37 ± .11, with reduced penetrance of .74 ± .2. Segregation ratios of .38 for one normal × normal mating (offspring of II-11 and II-12) and .43 for one normal × affected mating (offspring of II-3 and II-4), along with vertical transmission of clinical and EEG traits, best fit an autosomal dominant mode of inheritance with reduced penetrance. Power calculations (Ott 1989; Weeks et al. 1990) and

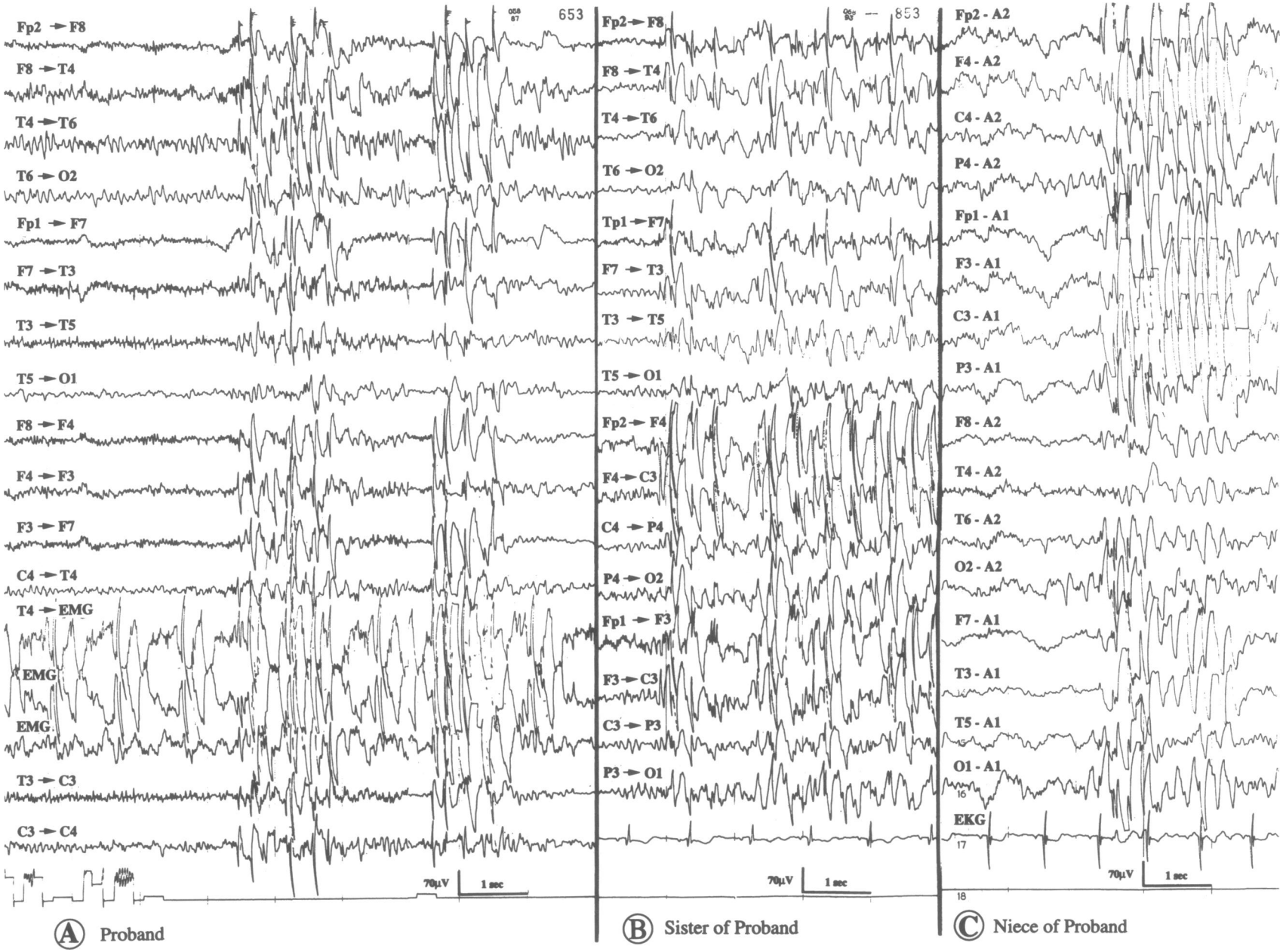


Figure 2 EEG 3.5–6-Hz diffuse multispike wave complexes in the proband (age 53 years), proband's sister (age 45 years), and proband's niece (age 8 years)

linkage analysis (Ott 1989) were therefore carried out, under the assumption of autosomal dominant transmission with 70% penetrance.

Genotypes for the pedigree were then simulated by use of the program SLINK (Weeks et al. 1990) with 1,000 replicates for each analysis. Under a 10-allele system of marker with equal frequencies and homogeneity, SLINK showed that linkage with JME or multispike wave complexes should be detected with average Z_{max} values of 3.994 and 3.110 ($\theta_{m=f}$ .000) under autosomal dominant inheritance with 90% and 70% penetrance, respectively. When clinically asymptomatic members with EEG multispike wave complexes were considered unaffected (the narrow diagnostic model), lod scores did not reach significant values, and the average Z_{max} was 1.46 ($\theta_{m=f}$ .000) under autosomal dominant inheritance with 30% penetrance.

2. *Classic JME in Seven Small Multiplex JME Pedigrees*

We validated the clinical and EEG status of seven families that were ascertained through a JME patient (five females and two males) and whose family members had either JME or EEG 3.5–6-Hz multispike wave complexes (fig. 3). The epilepsy phenotype of these seven small families are similar to that of the large LA-Belize family. Four of these pedigrees were included in our earlier reports (Greenberg et al. 1988*b*, 1993). The criteria for diagnosis of probands and family members were (1) MS started at age 8–20 years. GM clonic tonic-clonic seizures followed 1–2 years after onset of myoclonias at age 13–16 years in four probands. GM seizures appeared 6 years after myoclonias started, at age 8 years, in one patient. In one patient, GM attacks started 2 mo after myoclonias. In the proband of family 9, myoclonias and EEG 4–6-Hz multispike wave complexes were the only manifestations of seizures. (2) The interictal EEGs of the probands and affected family members showed diffuse and synchronous 3.5–6-Hz multispike wave complexes. (3) Intelligence and neurological status of all members were normal. (4) There was no history of head trauma associated with seizure onset, no family history of degenerative neurological disease, and no history of alcoholism or substance abuse. (5) None of the patients had MS that were stimulus sensitive only. (6) There were no PAs with 3-Hz spike and wave complexes in probands or affected family members. (7) There were no myoclonic absences or myoclonus absences or astatic-drop seizures in probands or family members.

For linkage analyses of these seven families, we used the diagnostic model where members symptomatic with JME and members without clinical epilepsy but with EEG 3.5–6-Hz polyspike wave complexes were classified as affected. Linkage analyses were carried out under the assumption of autosomal dominant inheritance with 70% penetrance. We classified member 6 of family 9 as unknown for linkage analysis, because she had transient benign and afebrile neonatal convulsions similar to those observed in member II-5 of the large LA-Belize family. Member 7 of family 57, member 3 of family 64, and member 21 of family 170 were considered unknown for linkage analysis, because they refused to have EEGs. Member 9 of family 57 and member 5 of family 159 had epileptiform but nonspecific diffuse spike and sharp wave formations and were thus considered unknown for linkage analysis.

3. *Nine Small Multiplex JME Pedigrees Atypical for Having PAs and 3-Hz Spike Wave Complexes*

We also studied the clinical and EEG states of nine families that had been ascertained through a patient with JME plus PAs. Family members had JME without PAs, or JME with PAs, or GM seizures only. Clinically asymptomatic members could have EEG 4–6-Hz polyspike wave complexes or 3-Hz spike wave complexes (fig. 4). In addition to the rapid 3.5–6-Hz polyspike wave complexes, typical of JME, the EEGs of probands of families 6, 11, 67, and 152 also showed paroxysms pathognomonic for childhood absence, namely diffuse 3-Hz spike and wave complexes. PAs appeared at age 8–13 years and preceded myoclonias in five probands—by 3 years in three of them and by 7–10 years in two of them.

In the other four patients, absences appeared either at the same time as did MS (one patient) or 7–8 years later (one patient). Two of these nine probands had infantile febrile convulsions.

For linkage analyses of these nine families, we broadened the diagnostic model further by including, as affected, family members with other forms of idiopathic generalized epilepsies, such as GM only. We considered as unknown for linkage analysis any living or deceased family members whom we were unable to examine, such as those in families 6, 9, 11, 28, 49, 104, and 152. We also considered as unknown for linkage analysis those individuals with epileptiform diffuse spike and sharp wave formations, such as member 2 of family 67 and member 21 of family 153.

Genotyping.—We isolated high-molecular-weight genomic DNA, using the standard phenol-chloroform method (Perbal 1988), and we followed the procedures of Weber and May (1989) in typing 146 STRPs. We used 20–30 ng of each patient's DNA, as template in a 10-μl PCR volume typically containing 1.5 mM $MgCl_2$, 250 μM each of dATP, dCTP, and dTTP, 2.5 mM of dGTP, 50 mM KCl, 10 mM Tris pH 8.3, 0.7 μCi α-P^{32}-dGTP (>1,000 Ci/nmol; Amersham), and 0.3 units *Taq* polymerase (Perkin Elmer Cetus). For amplification, samples were denatured for 6 min at 94°C, followed by 1.25 min annealing at 55°C or 60°C, and 0.25 min extension at 72°C, with a final extension step of 10 min at 72°C. After denaturation, a 3-μl sample of each

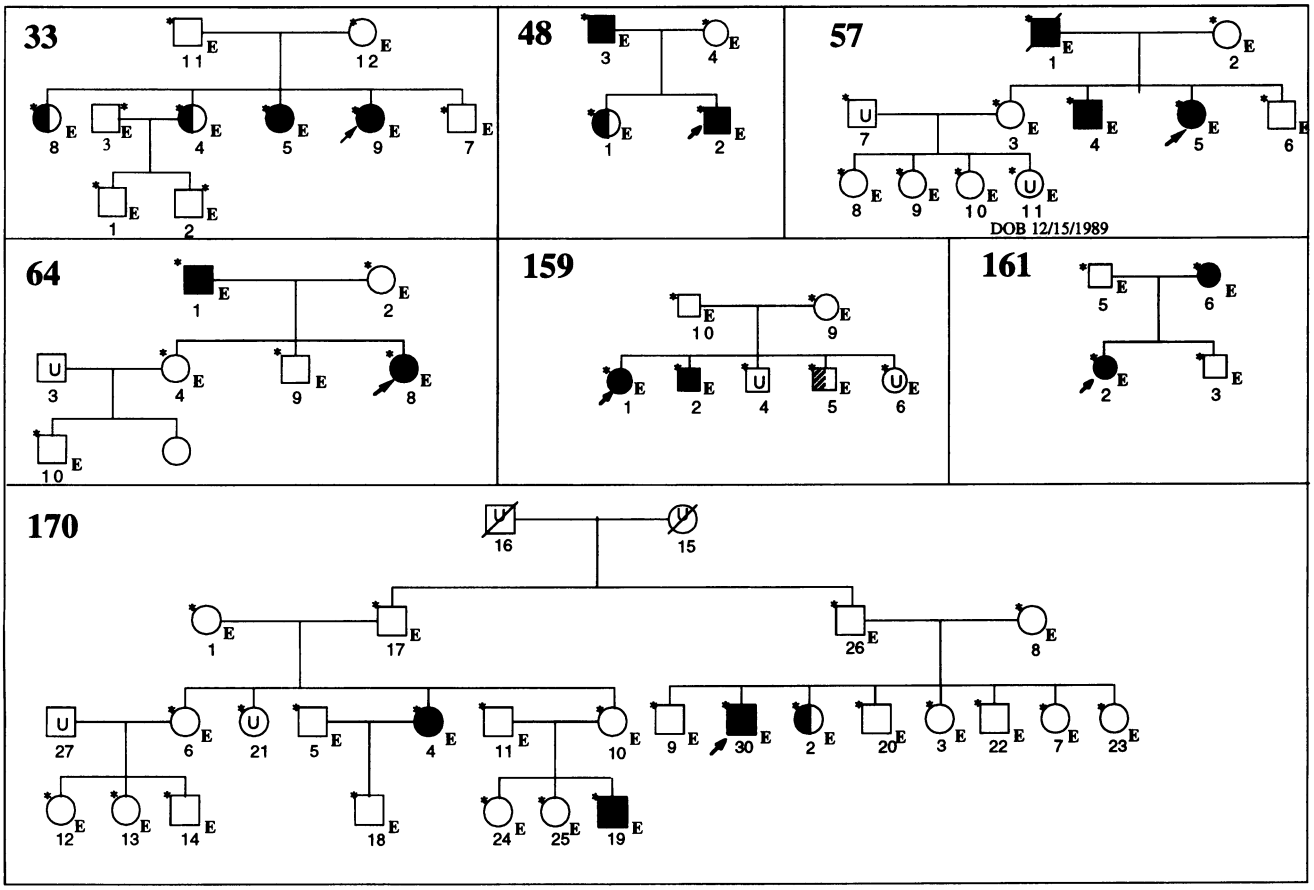


Figure 3 Seven multiplex pedigrees with classic JME. The completely blackened symbols represent persons with classic JME (no PAs); the half-blackened symbols represent asymptomatic family members whose EEGs show 3.5–6-Hz multispike wave complexes; and the half-hatched symbol represents an asymptomatic person whose EEG shows nonspecific but epileptiform bilateral and diffuse 4–7-Hz spike and sharp wave formations. An asterisk (*) denotes that DNA is available; an "E" indicates that an EEG was performed; and a "U" indicates that the affectedness status during linkage analyses was considered to be unknown.

reaction was immediately electrophoresed on prewarmed polyacrylamide gels (6.5% polyacrylamide/7.65 M urea acrylamide) for 2.75 h at 70 W. After electrophoresis, dried gels were exposed to Kodak XAR-5 or Fuji film. All sequences and allele frequencies of microsatellite were available from Genome Data Base (GDB) and were used for genotyping and linkage analyses. The majority of STRPs were (dC-dA)-(dG-dT) dinucleotide-repeat polymorphisms obtained from Research Genetics, as provided by the laboratories of Weissenbach et al. (1992) and Weber and May (1989). To maintain data integrity, consistencies were checked regularly and were insured by dual reading and scoring of genotypes on the autoradiographs. Interpretation and scoring of genotypes were done blind to the disease status of individuals being genotyped. Clinical epileptologists validated the clinical and EEG status of family members before microsatellite genotyping. EEGs were read and interpreted without knowledge of the person's name. Input and output of linkage analyses were also reviewed by two individuals.

Linkage analysis.—Pairwise and multipoint linkage analyses (Ott 1974) were performed by using the MLINK and LINKMAP routines of the LINKAGE program package, version 5.10. Lod scores were calculated at sex-averaged recombination frequencies of .000, .001, .01, .05, .10, .20, .30, and .40. Lod scores did not change appreciably when calculated with gene-frequency estimates of .001–.2.

Results

Myoclonic and GM Convulsions and EEG 3.5–6-Hz Multispike Wave Complexes Linked to Chromosome 6p21.2-p11 STRPs in the Large LA-Belize Family

After computer simulation (Weeks et al. 1990) determined an average Z_{max} of 3.110 ($\theta_{m=f}$ = .000) under autosomal dominant inheritance and 70% penetrance

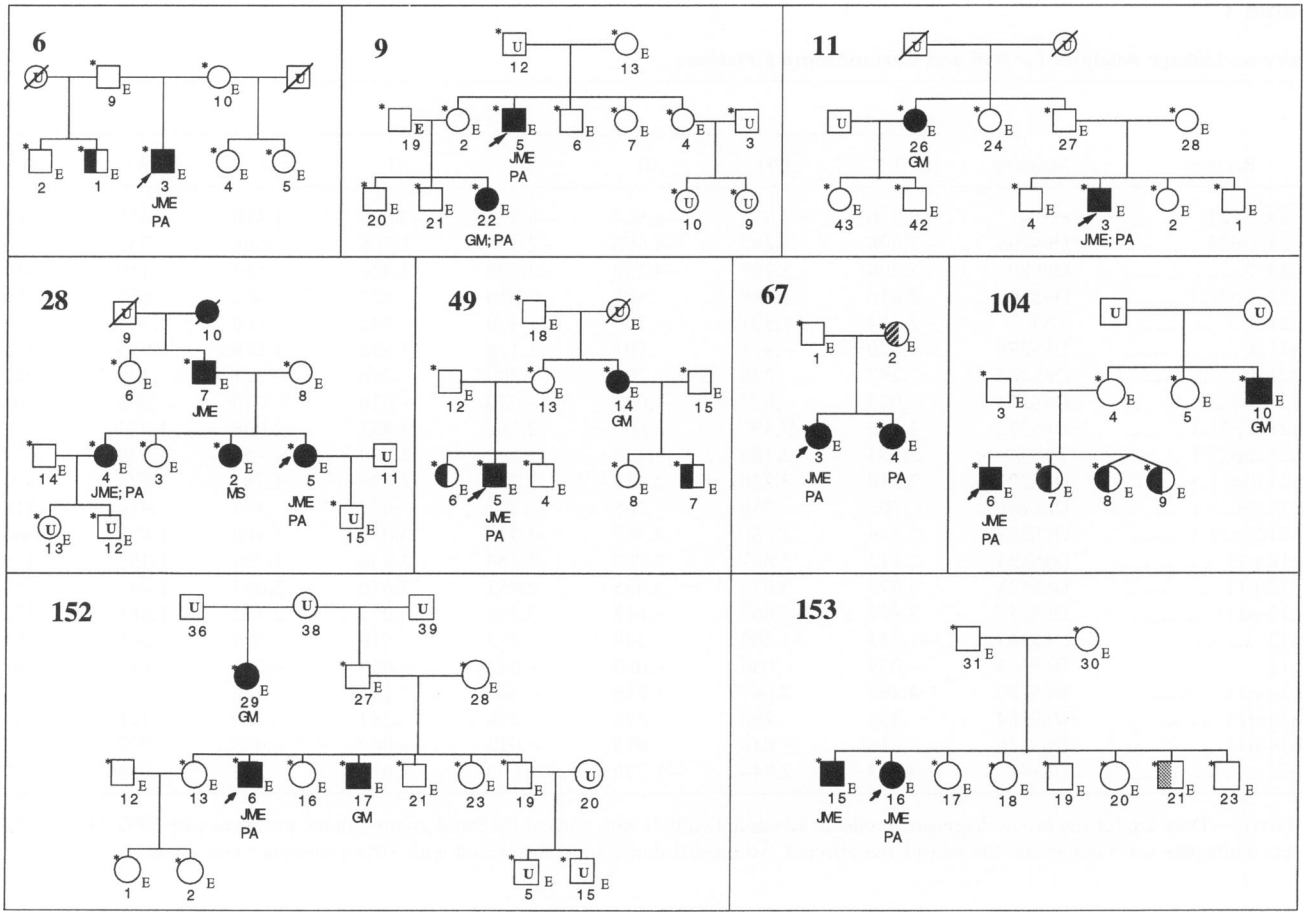


Figure 4 Nine multiplex pedigrees of patients with JME with PAs. The fully blackened symbols represent persons with idiopathic generalized epilepsies; the half-blackened symbols represent asymptomatic persons with 3-Hz spike wave complexes and/or 3.5–6-Hz multispike wave complexes in the EEGs; the half-hatched symbol represents an asymptomatic person with nonspecific but epileptiform diffuse spike-sharp slow wave formation in the EEG; and the half-shaded symbol represents a person with nonspecific, nonepileptiform but abnormal paroxysmal EEGs with diffuse 1–4-Hz slowing. Other symbols are as in fig. 3.

and after correction for the number of diagnostic models tested ($\log_{10}$ of 2), we projected a threshold lod score of 3.3 for linkage (Ott 1974; Risch 1991). Because exclusionary lod scores were reported in two separate studies of JME families (Liu et al. 1992; Rees 1992) during the First International Workshop on Chromosome 6, we used four STRPs from the HLA region and 142 STRPs from representative regions of the human genome (Weber and May 1989; Weissenbach et al. 1992), as a screening set during pairwise analyses with the clinical phenotypes of convulsions or EEG polyspike wave complexes.

We detected weak evidence for linkage with D6S276 on chromosome 6p21.3, with lod scores of 1.228 ($\theta_{m=f}$ = .20) and 1.295 ($\theta_{m=f}$ = .1). Because of these results, because STRPs telomeric to HLA excluded linkage, because lod scores did not reach the threshold for significance in other STRPs screened, and because more and new microsatellite markers centromeric to HLA became available (Weissenbach et al. 1992; Gyapay et al. 1994), we proceeded to genotype with more markers in chromosome 6p, centromeric to HLA. TCTE1 (lod score 2.18 at $\theta_{m=f}$ = .001), D6S282, D6S271, D6S269, and D6S294 gave positive but nonsignificant pairwise lod scores when tested independently with the JME locus (table 1). We obtained significant Z_{max} values, 3.564–3.560 with D6S272 and of 3.672–3.667 with D6S257, with $\theta_{m=f}$ = .00–.001. We also obtained a Z_{max} of 3.079–3.075 ($\theta_{m=f}$ = .00–.001) with D6S428, which was below the projected threshold for significance. When the female:male ratio (1.65 for chromosome 6) for recombination fractions is incorporated into the pairwise analysis, Z_{max} values for D6S257, D6S428, and D6S272 did not significantly change. Table 1 summarizes the two-point data over a range of recombination fractions for chromosome 6 markers used in the study.

Because other linkage-mapping studies on JME had considered family members with non-JME forms of idio-

Table 1

Pairwise Linkage Analyses for JME and Chromosome 6 Markers

Region	Marker	Z_{max} at θ = .0	.001	.01	.05	.1	.2	.3	.4
6p24.2-p23	F13A1	−9.570	−8.370	−6.524	−4.520	−3.068	−1.490	−.659	−.202
6p24.2-p23	D6S296	−7.008	−6.297	−4.690	−2.578	−1.596	−.668	−.232	−.036
6p23	D6S89	−5.200	−5.107	−4.250	−2.333	−1.426	−.565	−.159	.010
6p22.2-p21.3	D6S105	−2.610	−1.488	−.483	.310	.637	.802	.676	.389
6p21.3	TNF	−2.414	−1.301	−.307	.450	.742	.860	.704	.399
6p21.3	D6S276	−2.030	−.471	.505	1.126	1.295	1.228	.915	.465
6p21.3	D6S265	.297	.298	.299	.300	.286	.221	.129	.042
6p21.3	D6S273	−.023	−.023	−.023	−.020	−.016	−.010	−.005	−.001
6p21.2-p21.1	D6S282	2.596	2.597	2.600	2.562	2.432	2.008	1.434	.756
6p21.2-p21.1	TCTE1	2.183	2.185	2.196	2.190	2.100	1.759	1.270	.674
6p21.2-p21.1	D6S271	2.218	2.220	2.232	2.228	2.136	1.782	1.278	.673
6p21.2-p21.1	D6S269	.769	.768	.765	.742	.699	.573	.410	.216
6p21.2-p21.1	D6S272	3.564	3.560	3.517	3.315	3.036	2.400	1.671	.866
6p12-p11	D6S294	2.812	2.807	2.768	2.584	2.339	1.799	1.193	.539
6p12-p11	D6S428	3.079	3.075	3.035	2.851	2.610	2.081	1.483	.798
6p12-p11	D6S257	3.672	3.667	3.618	3.395	3.095	2.432	1.686	.872
6q13	D6S313	−1.753	−1.203	−.340	.293	.510	.598	.505	.301
6q12	D6S254	−.054	−.054	−.052	−.043	−.034	−.022	−.013	−.007
6q14-q15	D6S280	−4.498	−3.661	−1.748	−.367	.145	.426	.343	.125
6q14-q15	D6S284	.295	.296	.298	.298	.284	.220	.128	.041
6q14-q15	D6S286	−.016	−.016	−.015	−.012	−.009	−.005	−.002	−.005
6q23	D6S87	−3.713	−2.744	−1.770	−1.003	−.634	−.254	−.068	.009

NOTE.—Data are for the broad diagnostic model in which individuals with clinical JME and asymptomatic members with EEG diffuse 3.5–6-Hz multispike wave complexes are considered affected. Autosomal dominant transmission with 70% penetrance was assumed.

pathic generalized epilepsies to be affected (Durner et al. 1991; Weissbecker et al. 1991; Whitehouse et al. 1993), we examined whether the phenotype of member II-5 was part of the JME syndrome, by changing her status to "affected" during linkage analyses. With IV-3 and IV-1 still remaining in the "unknown" affected status, pairwise Z_{max} values for D6S257 rose to 4.076 and 4.071 ($\theta_{m=f}$ = .000–.001), and those for D6S272 rose to 3.969 and 3.963 ($\theta_{m=f}$ = .000–.001). When family member II-5 was considered unaffected, lod scores did not drop below significance.

We also tested for linkage under the most stringent assignments for affectedness. We considered the father of the proband unknown for linkage analysis and designated family member II-5 as unaffected. Under these conditions, lod scores reached the traditional threshold for linkage (Z_{max} 3.01 for D6S272 and 3.0 for D6S257, both at $\theta_{m=f}$ = .001) but were slightly less than the projected threshold of 3.3.

When clinically asymptomatic family members who have diffuse multispike wave complexes in their EEGs were not considered affected and we assumed autosomal dominant inheritance with 30% penetrance, we still obtained positive lod scores ($\theta_{m=f}$ = .001) of 1.232 with D6S282, 1.238 with D6S271, 1.45 with D6S272, and 1.478 with D6S257. With family member II-5 considered affected, lod scores rose for D6S272, D6S428, and D6S257 (lod scores 1.880–1.877, 1.286–1.284, and 1.919–1.915, respectively, with $\theta_{m=f}$ = .000–.001).

Table 2

Various Orders for JME Gene and Markers

Order	Z_{max}
D6S271–.022–TCTE1–0–JME	2.7060
JME–.025–D6S271–.022–TCTE1	2.6765
D6S272–0–JME–.040–D6S271	3.2674
D6S294–0–JME–.065–D6S272	3.5643
D6S257–0–JME–.018–D6S294	3.6722
JME–0–D6S257–.078–D6S272	3.6722
D6S313–.058–D6S257–0–JME	3.3698

NOTE.—Overlapping three-point multipoint linkage analyses were carried out by moving the JME locus through seven DNA markers in the region of chromosome 6p21.2-6q13. The θ values between each pair of markers, as well as the genetic map used in the multipoint analyses (i.e., D6S313–.058–D5S257–.008–D6S428–.010–D6S294–.065–D6S272–.040–D6S271–.022–TCTE1), are based on both the consensus map reported by the Human Genome Chromosome 6 Workshop (Volz et al. 1994), held in Berlin in 1993, and the Généthon Human Genetic Linkage Map (Gyapay et al. 1994).

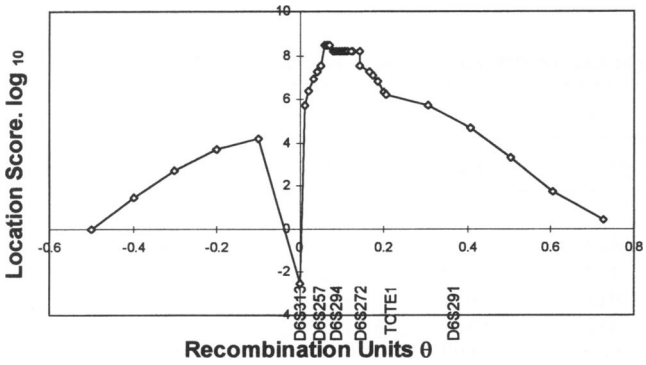


Figure 5 Multipoint location scores as a function of map distance (in recombination units).

However, as anticipated by computer simulation studies, lod scores did not reach the conventional threshold of 3.0.

We attempted to estimate the position of the JME locus in relation to markers proximal to TCTE1 (chromosome 6p21.2) and D6S313 (chromosome 6q13) (Lathrop et al. 1984). LINKMAP supported results of pairwise analyses but could not find a most probable order for the disease locus in relation to D6S257–D6S294–D6S272 (see table 2), because no recombinations were detected between JME and these DNA markers (Gyapay et al. 1994; Volz et al. 1994). Two orders—JME-D6S257-D6S272 (interval θ's .000 and .078, respectively) and D6S257–JME–D6S294 (interval θ's .00 and .018, respectively)—obtained the highest lod score, 3.6722. The next highest lod score obtained was 3.5643, with interval θ at .000 and .065, for the order of D6S257–JME–D6S272. These multipoint orders were not significantly different in their probabilities, since the next three likely orders had significant lod scores of 3.5643, 3.3698, and 3.2674 (see table 2).

Three-point location scores were also calculated, in order to determine the most likely location of the JME locus within the candidate region surrounding and including D6S257, D6S294, and D6S272 (see fig. 5). The resulting location scores peaked within the 7-cM interval defined by D6S257, D6S294, and D6S272. However, if we drop 3 log-base-10 lod units below the maximum, we identify a support interval considerably larger (35 cM), flanked by D6S313 and D6S291. Such a conservative step is considered prudent, since the magnitude of errors in approximating location scores is unknown (Lange and Sobel 1991).

We next proceeded to analyze the 13 marker haplotypes of 34 family members with D6S105 and D6S257 as the most telomeric and centromeric loci, respectively. We observed one informative recombination between D6S276 and D6S273 in family member III-8, who is clinically affected. This individual shares with members affected with convulsions or EEG multispike waves the JME-bearing haplotype, which includes D6S276–D6S257 and positions the JME region within or below HLA (see fig. 1). Three informative recombinations occurred in clinically and EEG-unaffected members III-1, III-2, and III-9. In family members III-2 and III-9, a recombination suggested the locus to be below D6S265. A recombination in family member III-1, between D6S269 and D6S271, narrowed the locus region further, to 9–14 cM. The significance of a recombination in a normal individual is always tenuous, both because such a normal individual could develop the disease at a later date and because of incomplete penetrance. In our present JME family, onset of clinical JME and EEG multispike wave complexes was at age 10 years, and concordance for age at onset within sibships was generally ±3 years. In other JME families, the clinical disease and EEG multispike wave complexes rarely start after age 18 years. Consequently, since these three individuals are older than age 20 years, the probability that they will develop JME in the future is very unlikely.

Myoclonia, GM, and EEG Multispike Wave Complexes Also Linked to Chromosome 6p21.2-p11 STRPs in Seven Small Classic JME Pedigrees without PAs

To determine if the same chromosome 6p21.2-p11 STRPs also have a role in smaller classic JME families, we studied families of patients whose clinical phenotypes were similar to that of the LA-Belize family. As in the LA Belize family (in which there were 3 affected females vs. 4 affected males), we noted no significant sex preponderance in clinically affected members of the smaller families (10 affected females vs. 8 affected males). However, among EEG-affected individuals who are clinically asymptomatic, only females were affected with the EEG polyspike wave trait (three females vs. no males in both LA-Belize and smaller families).

We detected possible evidence of linkage with D6S272 on chromosome 6p21.2, with a lod score of 2.973 ($\theta_{m=f}$ = .000) after we pooled and summed the individual lod scores of each small pedigree, assuming that homogeneity existed in these seven families. Table 3 shows the results of pairwise linkage analyses between convulsions, the EEG trait, and chromosome 6p21.2-p11 markers. Lod scores reached the threshold for significance with chromosome 6p11 STRPs, namely 4.726 for D6S294 and 3.351 for D6S257, at $\theta_{m=f}$ = .000 (see table 3).

Exclusion of Linkage between Chromosome 6p21.2-p11 STRPs and JME Atypical for Having PAs or 3-Hz Spike and Wave Complexes

We proceeded next to screen with chromosome 6p21.2-p11 STRPs nine JME families atypical for having PAs or 3-Hz spike and wave complexes. Four of

Table 3

Pairwise Z_{max} Values between JME and Each of Three Markers in Eight Classic JME Families with No PAs or 3-Hz Spike Wave

Marker and Family	Z_{max} at θ =							
	.000	.001	.010	.050	.100	.200	.300	.400
D6S272:								
33	.940	.938	.926	.868	.785	.589	.356	.119
48	.300	.299	.292	.257	.214	.133	.064	.017
57	−.018	−.018	−.018	−.016	−.014	−.011	−.007	−.004
64	.411	.410	.400	.357	.303	.195	.098	.027
159	−.000	−.000	−.000	−.000	−.000	−.000	−.000	−.000
161	.187	.186	.181	.157	.128	.077	.036	.009
170	1.486	1.483	1.455	1.327	1.164	.828	.484	.169
J-1	3.564	3.560	3.517	3.315	3.036	2.400	1.671	.866
Total	6.870	6.858	6.752	6.264	5.615	4.210	2.701	1.203
D6S294:								
33	.940	.938	.926	.868	.785	.589	.356	.119
48	.300	.299	.292	.257	.214	.133	.064	.017
57	.755	.753	.739	.674	.590	.412	.227	.068
64	.411	.409	.399	.354	.297	.186	.087	.020
159	−.017	−.017	−.016	−.014	−.011	−.006	−.003	−.001
161	.187	.186	.181	.157	.128	.077	.036	.009
170	2.150	2.146	2.105	1.921	1.687	1.210	.723	.253
J-1	2.683	2.679	2.639	2.456	2.212	1.673	1.068	.418
Total	7.409	7.394	7.264	6.673	5.903	4.273	2.559	.903
D6S257:								
33	.673	.672	.661	.610	.538	.378	.207	.061
48	.300	.299	.292	.257	.214	.133	.064	.017
57	.755	.753	.739	.673	.588	.408	.223	.065
64	.411	.409	.399	.354	.297	.186	.087	.020
159	−.335	−.333	−.316	−.248	−.183	−.093	−.039	−.009
161	.187	.186	.181	.157	.128	.077	.036	.009
170	1.361	1.359	1.340	1.251	1.127	.839	.511	.184
J-1	3.672	3.667	3.618	3.394	3.094	2.431	1.685	.871
Total	7.023	7.012	6.914	6.447	5.804	4.357	2.774	1.217

NOTE.—Autosomal dominant transmission with 70% penetrance was assumed.

the nine pedigrees had enough family information by themselves so that each of them had lod scores that reached significance for exclusion of linkage to STRPs in chromosome 6p21.2-p11, namely D6S294 and D6S257, at $\theta_{m=f} = 0–.001$. Consequently, summed lod scores in these nine families were −11.572 for D6S272, −12.799 for D6S294, and −12.239 for D6S257, all at $\theta_{m=f} = .00$ (see table 4).

Discussion

The characteristic feature and often-presenting complaint of JME, without which a correct diagnosis cannot be made, is adolescent-onset myoclonic jerks that are bilateral, single or repetitive, and mostly symmetric. Generalized tonic-clonic seizures usually either appear simultaneously with MS or follow within 2–10 years. In the course of illness, as tonic-clonic seizures are controlled with drug treatment, myoclonias break through because of sleep deprivation, alcohol intake, or severe fatigue. These characteristic features of JME were present in all of the patients whom we studied.

For classifying the common idiopathic epilepsies, we emphasized, in 1983, the importance of separating childhood absences of the pyknoleptic variety, with its many daily attacks often numbering into the hundreds, from the sporadic and rare (spanioleptic) absences observed in JME (Delgado-Escueta et al. 1983). Impairment of consciousness is not as deep, while comprehension and expression of speech may remain intact in the random and rare spanioleptic absences in JME. We noted that the syndrome of remitting childhood-absence epilepsy was different from the syndrome of persisting childhood-absence epilepsy. We also cautioned that rare forms of myoclonic absences and myoclonus absences should not be mixed with JME. In our present studies, we first used one relatively large, LA-Belize family to provide independent proof that a genetic locus for classic JME and its EEG polyspike wave trait is proximal to HLA in chromosome 6p21.2-p11. This single large fam-

Table 4

Pairwise Z_{max} Values for JME and Each of Three Markers in Nine JME Families Atypical for Having PAs and/or 3-Hz Spike Wave

Marker and Family	Z_{max} at θ = .000	.001	.010	.050	.100	.200	.300	.400
D6S272:								
6	−.012	−.012	−.012	−.010	−.008	−.004	−.002	−.000
9	−.670	−.665	−.620	−.467	−.340	−.182	−.089	−.032
11	.021	.021	.021	.020	.017	.005	−.002	−.002
28	−2.610	−2.182	−1.363	−.695	−.417	−.172	−.064	−.014
49	.150	.150	.146	.129	.108	.068	.034	.011
67	−2.586	−2.182	−1.376	−.716	−.442	−.193	−.076	−.018
104	−2.135	−2.129	−1.980	−1.210	−.734	−.304	−.114	−.026
152	−3.515	−3.378	−2.561	−1.406	−.874	−.386	−.154	−.039
153	−.217	−.216	−.212	−.191	−.163	−.102	−.048	−.012
Total	−11.572	−10.594	−7.956	−4.546	−2.853	−1.270	−.514	−.134
D6S294:								
6	.487	.486	.475	.428	.366	.242	.124	.035
9	−3.029	−2.350	−1.444	−.774	−.501	−.257	−.134	−.055
11	−2.453	−2.351	−1.872	−1.181	−.798	−.381	−.155	−.037
28	−2.341	−1.855	−1.021	−.406	−.191	−.050	−.013	−.003
49	−2.657	−2.294	−1.508	−.836	−.546	−.274	−.137	−.054
67	−.300	−.298	−.283	−.225	−.167	−.086	−.036	−.009
104	−2.135	−2.219	−1.980	−1.210	−.734	−.304	−.114	−.026
152	−.038	−.037	−.023	.022	.054	.074	.063	.037
153	−.332	−.331	−.317	−.260	−.200	−.108	−.047	−.011
Total	−12.799	−11.249	−7.974	−4.443	−2.717	−1.144	−.448	−.123
D6S257:								
6	.487	.486	.475	.428	.366	.242	.124	.035
9	−3.541	−2.372	−1.403	−.723	−.445	−.196	−.078	−.019
11	−2.452	−2.062	−1.268	−.625	−.368	−.150	−.056	−.013
28	.187	.186	.181	.157	.129	.077	.036	.009
49	−2.991	−2.439	−1.562	−.868	−.566	−.283	−.141	−.056
67	−.300	−.298	−.283	−.225	−.167	−.086	−.036	−.009
104	−2.135	−2.129	−1.980	−1.210	−.734	−.304	−.114	−.026
152	−1.278	−1.259	−1.118	−.749	−.499	−.226	−.087	−.020
153	−.217	−.216	−.212	−.191	−.163	−.102	−.048	−.012
Total	−12.239	−10.103	−7.169	−4.006	−2.447	−1.028	−.399	−.111

NOTE.—Autosomal dominant transmission with 70% penetrance was assumed.

ily allowed us to perform linkage analyses without having to examine more than two diagnostic models (a narrow and a broad diagnostic model) and without having to change genetic parameters. We assessed only the mode of inheritance determined by calculations of affected siblings across mating types, namely autosomal dominant inheritance with 70% penetrance. This LA-Belize JME family belongs to the subset whose epilepsies consist mainly of classic JME (Commission on Classification and Terminology of the International League against Epilepsy 1989; Delgado-Escueta et al. 1994). We did not encounter in the LA-Belize family any members who are affected either with PAs as their sole epilepsy phenotype or with PAs in combination with GM seizures. In our present analysis of small pedigrees with classic JME, we also excluded families with PAs even when the latter had started after age 8 years, because PAs are more characteristic of childhood-absence epilepsy. Summed lod scores of small families with classic JME, by breaking the threshold for significance (lod scores 3.351–4.726 for D6S257 and D6S294 [$\theta_{m=f}$ = .000), lent additional support for the presence of an epilepsy locus in chromosome 6p21.2-p11. Total lod scores obtained from summing values from the large LA-Belize family and the six small families with classic JME were 7.02 for D6S257, 6.870 for D6S272, and 7.409 for D6S294 ($\theta_{m=f}$ = .000).

We pursued further the role of PAs in JME, by contrasting linkage results in classic JME versus linkage results in JME atypical for having PAs. In contrast to small families with classic JME, small families with JME and PAs had summed lod scores that excluded linkage to chromosome 6p21.2-p11. In fact, individual lod scores were exclusionary (<−2.0 at $\theta_{m=f}$ = .000-.001) for

D6S294 and D6S257 in chromosome 6p21.2-p11 in each of four small families atypical for having PAs. Mixing JME families atypical for having PAs with classic JME families would have made us miss linkage with STRPs. Such phenotypes of PAs only or PAs with GM were present in affected members of JME families reported from the United Kingdom and Sweden.

Pairwise Z_{max} values and one recombination event in the LA-Belize family prove that a mutation segregates with convulsions and the EEG polyspike waves. $\theta_{m=f} < .001$ provided strong evidence for very tight linkage between JME and two DNA markers spanning 7.0 cM, namely D6S272 and D6S257. Three-point linkage analysis did not reveal a significantly more probable order, because no recombinations between epilepsy and linked markers were detected in this family. Although a clear ordering of the disease locus with respect to D6S272 and D6S257 could not be resolved, Z_{max} values were obtained when JME was placed in the close vicinity of D6S257 ($\theta_{m=f} = .000$). Analysis of 13 marker haplotypes in chromosome 6p, which include D6S105 and D6S257 as the most telomeric and centromeric loci, respectively, indicated one recombination event between D6S276 and D6S273 in one affected family member (III-8) and suggested that the epilepsy locus is within or centromeric to HLA. When this sole recombination in family member III-8 is correlated with results of pairwise analyses that tightly link convulsions and EEG multispike wave complexes to centromeric DNA markers D6S257 and D6S272, our overall results in the LA-Belize family could be interpreted to mean that the JME region is centromeric to HLA. We did not find any recombinations in affected members of the small families with classic JME. Studies looking for recombination events and large families that segregate the convulsive-epilepsy syndrome of JME and its EEG traits and more polymorphic microsatellites in the chromosome 6p21.2-p11 region are needed to resolve the epilepsy gene/marker order. Large pedigrees ascertained through a proband with JME and segregating only the EEG trait of 3.5–6-Hz multispike wave complexes in asymptomatic family members are also needed, to determine if the chromosome 6p21.1-p11 locus is a genetic site primarily for the EEG trait, clinical epilepsy, or both. We should also seek an explanation for why only asymptomatic females or a preponderance of asymptomatic females are affected with the EEG polyspike wave complex.

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