

JUVENILE MYOCLONIC EPILEPSY (JME) MAY BE LINKED TO THE BF AND HLA LOCI ON HUMAN CHROMOSOME 6

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Although certain forms of epilepsy have long been suspected to be inherited, heterogeneity has made it difficult to find the genes responsible for any subtypes. We found that families ascertained through patients with juvenile myoclonic epilepsy show linkage with the BF and HLA loci on human chromosome 6. There is some evidence that the locus may be outside the HLA complex and no evidence as yet of an association with any allele of the HLA complex.

Key words: juvenile myoclonic epilepsy, linkage, genetics, EEG, HLA, chromosome 6, myoclonia

INTRODUCTION

Although authors since ancient times have suggested that epilepsy is an inherited "syndrome," causal heterogeneity has made epilepsy difficult to study as a genetic disorder. In this report we show that grand mal and myoclonic epilepsy of adolescence (or juvenile myoclonic epilepsy) may be genetically linked to the BF and HLA markers on chromosome 6.

Juvenile myoclonic epilepsy (JME) is a non-progressive form of generalized epilepsy that begins in adolescence, afflicting otherwise normal individuals. The clinical traits are apparently life-long, so most patients have grand mal and myoclonic seizures in later years of life as well. These traits were originally described in Europe as a separate form of epilepsy by Janz and Christian [1957]. JME was recognized in North America by Delgado-Escueta [Delgado-Escueta, 1980; Delgado-Escueta et al., 1982; Delgado-Escueta and Enrile-Bacsal, 1984] and by Dreifuss [1983] and Asconapé and Penry [1984]. JME is thought to represent between 4 and 10% of all epilepsies. Although JME has characteristics common to many generalized epilepsies, it can be distinguished by two major characteristics: (1) the age-at-onset of seizures is between the 9th and 20th years of life (mean is 15 years) and (2) myoclonic jerks, which occur most often in the morning after awakening but can occur at any time, are present in all patients. Grand mal tonic-clonic or clonic-

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tonic-clonic seizures occur in 95% of patients. About 30% of patients have absence seizures. All untreated patients that we have studied have an interictal EEG trait (i.e., when not having a seizure), which consists of bilaterally symmetric diffuse 4-6 Hz multispikes-and-wave complexes. Ten percent also have classic diffuse 3 Hz spike-wave complexes. Of critical importance is the observation that these EEG abnormalities, diffuse paroxysmal theta rhythms, and mixtures of diffuse spikes, sharp waves, and theta rhythms can occur in some apparently unaffected relatives of JME patients [Tsuboi and Christian, 1973; Greenberg et al., 1988]. We considered these abnormal EEG traits found in clinically normal relatives as subclinical markers for at least part of the disease genotype.

DATA AND METHODS

Our data came from 24 families where the proband had JME. The criteria for diagnosis were: 1) age-of-onset of any seizure type between 8 and 20 years; 2) normal intelligence and normal neurological status; 3) myoclonic jerks which usually occur in the mornings or on awakening (but which can occur any time during the day) that do not invariably lead to major seizures; 4) no history of head trauma associated with the onset of seizures; 5) no family history of degenerative neurological disease; and 6) no history of alcoholism or other substance abuse. We also excluded those patients in whom clinical seizures could be induced by photostimulation. Those patients who showed abnormalities on photostimulation only in the EEG record were not excluded.

We hoped that by strict adherence to these criteria we could eliminate other epilepsy syndromes from our data.

We observed that 95% of JME patients have tonic-clonic seizures and about 30% have absence [Greenberg and Delgado-Escueta, in preparation]. About 5% have *only* jerking as a seizure type. In our sample of 24 probands that were included in the linkage analysis, 10 had absence in addition to tonic-clonic seizures and three patients showed photosensitivity in the EEG record. None of the patients in the sample used for linkage analysis had *only* myoclonic jerks.

The data, obtained with informed consent, consisted of typing information on 25 phenotypic blood markers and of 30 to 60 minute EEG data from as many relatives as possible. Marker symbols are defined according to McAlpine et al. [1985].

Our data [Greenberg and Delgado-Escueta, in preparation] show that about 15% of sibs of JME patients have EEG traits without any clinical symptoms. These EEG traits consist of diffuse paroxysmal theta rhythms and mixtures of diffuse spikes and sharp waves mixed with the theta rhythms [Tsuboi and Christian, 1973; Greenberg et al., 1988]. These EEG traits, when they occur at all, are seen frequently in a one-hour EEG record. There was seldom any ambiguity about their presence. In the sample used for the linkage analysis, out of 48 sibs who had EEGs, 7 clinically normal sibs had abnormal EEGs (14.3%) and an additional 3 sibs had JME. Two parents (out of 41 tested) had abnormal EEGs.

For the purposes of the linkage analysis, we classified as "affected" patients with JME and also asymptomatic individuals who had an EEG with diffuse 4-6 Hz multispikes-and-wave complexes, or with paroxysmal, generalized theta (4-7 Hz) rhythms, or mixtures of generalized spikes, sharp-waves and theta rhythms. Most of the EEG abnormalities we observed (7 out of 9) among asymptomatic relatives were of this last type. Two out of 9 unaffected relatives with abnormal EEGs showed the spike-and-wave trait similar to that seen in JME patients.

Linkage analysis assesses the likelihood of genetic linkage between a known genetic marker and a trait or disease whose chromosome location is unknown. We used the program LIPED [Ott, 1975] to perform the calculations.

Segregation analysis [Greenberg et al., 1988] indicated that the most favorable model was a two-locus model, with one locus recessive and the other either dominant or recessive. Unfortunately, there was no program available to us which does the linkage calculations taking into account the case where the trait is the result of two epistatically-interacting loci. The closest acceptable model available was to assume recessive inheritance with reduced penetrance. In a previous work, we used simulation to test the effect of assuming a single locus model for linkage analysis even though the data actually represented a two-locus disease [Greenberg and Hodge, 1988]. The results of that work showed that assuming a single-locus model with random reduced penetrance for linkage analysis when the disease is actually the result of two epistatically-interacting loci does not distort the results of linkage analysis. Our simulation work also showed that, when doing linkage analysis on a trait that is the result of two epistatically-interacting loci, the inheritance of the *trait per se* is not the critical concern. Of greater importance for detecting linkage is the inheritance at the locus linked to the marker. Thus, for linkage analysis, we first assumed recessive inheritance with 60% penetrance and a gene frequency of 0.1. We then did the analysis assuming a fully penetrant recessive and also a dominant model, which we tested since the segregation analysis could not rule out dominant inheritance for one of the two possible loci.

Since we classified asymptomatic relatives with abnormal EEGs as affected, we were actually testing the combined EEG and epilepsy traits. Therefore, to estimate gene frequency, we needed to estimate the population prevalence of the types of EEG abnormalities that are observed. A number of EEG studies of the general population estimate the prevalence of "paroxysmal EEG abnormalities" at between 1 and 6% [Christiani, 1979; Chatrian, 1976; Oberholz et al., 1975]. These include not only diffuse 3-6 Hz multispikes-and-wave complexes, but also focal abnormalities, which we classify as unrelated to the JME syndrome. Focal abnormalities were not counted as abnormal for this analysis. We also assumed several different gene frequencies for the linkage analysis.

We also ran the analyses assuming that the EEG trait represented a trait different from JME. In that case, those subjects without epilepsy but with abnormal EEG traits were classified as "normal."

Results are in terms of lod scores [Morton, 1955]. The "lod score" or "log of the odds" for linkage, expresses the odds in favor of linkage between the trait and the marker versus no linkage. A lod score of 3, or 1000:1 odds in favor of linkage, is generally accepted as sufficient evidence that linkage exists if the mode of inheritance is known.

We calculated the likelihoods for values of the recombination fraction, $\theta = 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, \text{ and } 0.5$.

RESULTS

Of the standard blood markers, the highest lod score occurred with BF (properdin factor B), which is located on chromosome 6 (Table I). Of the 24 families included in the analysis, 11 families were informative for BF. These lod scores summed to 1.66 when the inheritance was assumed to be recessive and the penetrance was 0.6. The value of the lod score under the assumption of full penetrance and a recessive mode of inheritance was 1.52.

At least 7 markers gave significant negative lod scores (less than -2 or 1:100 odds *against* linkage): C3, GPT, HP, MNSS, PBG, PGM1, and RH for some values of θ . Several others gave lod scores in the range of -1.0 to -2.0 under the assumption of tight linkage: ADA, GC, ESD, KKP, Duffy, and TF (Table II). GLO1 had a noticeably negative lowest lod score (-1.2) but it also has a highest lod score close to unity. This is not inconsistent with the finding of linkage of JME to BF (see below).

TABLE I. Lod Scores for BF Alone and Combined BF-HLA Information

Penetrance	BF alone		HLA + BF	
	LOD score	θ (male,female)	LOD score	θ (male,female)
1	1.52	(.20, .01)	3.04	(.01, .10)
0.6	1.66	(.01, .01)	3.03	(.01, .05)

θ is the recombination fraction, calculated using different male and female recombination rates.

One other marker, JK, gave a lod score of over 1.0.

BF is located in the middle of the HLA region. Since HLA haplotypes are usually inherited together, HLA haplotype data can be substituted for BF data in the linkage analysis. HLA loci, when used as a linkage markers, are almost always informative for linkage because of the large number of alleles at those loci. One can usually determine which haplotype came from which parent. We obtained HLA typing in 3 of our multiplex families (2 or more sibs affected) and substituted the HLA lod scores for the BF lod scores in those families. These results (Table I) give a lod score of greater than 3.0. The effect of varying the gene frequency was small. Lod scores for BF differed by less than 5% in the range of gene frequencies for the trait from 0.05 to 0.2. Changing the assumed penetrance from 0.6 to 1.0 resulted in little change in the lod score.

In order to see how much information for linkage is contributed by the abnormal EEG traits that we see in relatives, we did the analysis classifying those relatives with abnormal EEGs as being without the trait. Only those people with clinical JME were classified as affected. The lod score for BF became negative (< -2.0). The combined lod score for HLA-BF, after substituting the HLA lod score for the BF scores for those families for which we had HLA data, was -3.6 . This indicates no linkage of JME alone with the BF-HLA locus when the abnormal EEGs are classified as "normal."

We also examined the case where the inheritance was assumed to be dominant. It is generally accepted that misspecifying the mode of inheritance may *mask* the existence of linkage, but, with sufficient data, linkage will still be detected. When we ran the analysis assuming dominant inheritance for JME and the EEG traits, the lod score for BF was still 1.14 at an assumed penetrance of 0.3. (A penetrance of 0.3 is what must be assumed under a dominant model to explain the observed segregation ratio [Greenberg et al., 1988].) The corresponding value for the HLA locus was 1.24 for the 3 multiplex families for which we had HLA typing. Assuming a penetrance of 0.9 with a dominant model, the lod score was still 1.22 for BF and 1.46 for HLA.

DISCUSSION

Establishing that genetic linkage exists between a known marker and the EEG traits leaves little doubt that those EEG traits have a genetic basis. While mixtures of sharp waves, spikes and theta rhythms are generally accepted as abnormal patterns, the diffuse, paroxysmal theta rhythm seen in some relatives of JME patients has not generally been considered "epileptiform." Because of these previous EEG interpretations in the literature, we also performed linkage analysis classifying all EEGs from asymptomatic relatives as "normal," that is, we looked for linkage counting only those with clinical epilepsy as affected. The lod score for HLA-BF was negative (-3.6 for tight linkage) under this assumption. This indication of "no linkage" when the paroxysmal EEGs in unaffected

TABLE II. Summary of Significant LOD Scores and Selected Recombination Fractions for All Markers Under Recessive Inheritance with 60% Penetrance.

Name of marker	Highest LOD score	Recombination* fraction	Lowest LOD score	Recombination fraction
ABO	0.55		-0.51	
ACP	0.46		-0.23	
ADA	0.00		-1.10	
AK1	0.00		-0.28	
AMY2	0.00		0.00	
BF	1.66	(.01,.01)	0.00	
C3	0.00		-2.45	(.01,.01)
E2	0.00		0.00	
GALT	0.48		-0.16	
GC	0.28		-1.73	(.01,.01)
GLO1	0.91	(.50,.01)	-1.20	(.01,.50)
JK	1.08	(.01,.01)	0.00	
6PGD	0.00		0.00	
DUFFY	0.29		-1.30	(.01,.01)
ESD	0.32		-1.40	(.50,.01)
GPT	0.00		-2.60	(.01,.01)
HP	0.00		-2.90	(.01,.01)
MNSS	0.00		-7.70	(.01,.01)
KKP	0.00		-1.13	(.01,.01)
PBG	0.10		-2.14	(.01,.50)
PGM1	0.00		-3.50	(.01,.01)
PGP	0.00		-0.74	
PI	0.10		-0.60	
RH	0.00		-3.50	(.01,.01)
TF	0.15		-1.30	(.50,.01)

* (male θ , female θ)

relatives are considered "normal," together with the strong evidence in favor of linkage when they are considered part of the phenotype, forces us to conclude that the EEG paroxysms are related genetically to the epilepsy, whether they fit the generally accepted definition of "epileptiform" or not. The only reasonable alternative conclusion is that, if one insists that the EEG paroxysms in asymptomatic relatives have nothing to do with epilepsy, then the positive linkage result must be totally spurious. But, it is highly improbable that taking some random trait and defining it as related to the disease under study would lead to a positive linkage result, much less a statistically significant one. If our linkage result is correct, the significance of paroxysmal EEG traits, and perhaps the specificity of EEG traits in general, must be re-evaluated.

The finding of linkage of a disease with HLA immediately raises the question of whether the disease is associated with a specific allele or a haplotype of the HLA locus. If the JME locus lies within the HLA region, then JME will most likely be associated with an allele of the HLA system. We found no significant HLA association with JME in examining the HLA data on 22 of the probands, although such a small sample would not necessarily demonstrate a weak association. However, Durner et al. [1988] report no significant HLA association with JME using a sample of 90 patients.

Additional support for the idea that the locus lies outside the HLA region also comes from examining the HLA types of one of the multiplex families. In one family (designated EPO33), there were 4 affected sibs, two with clinical epilepsy and two with abnormal

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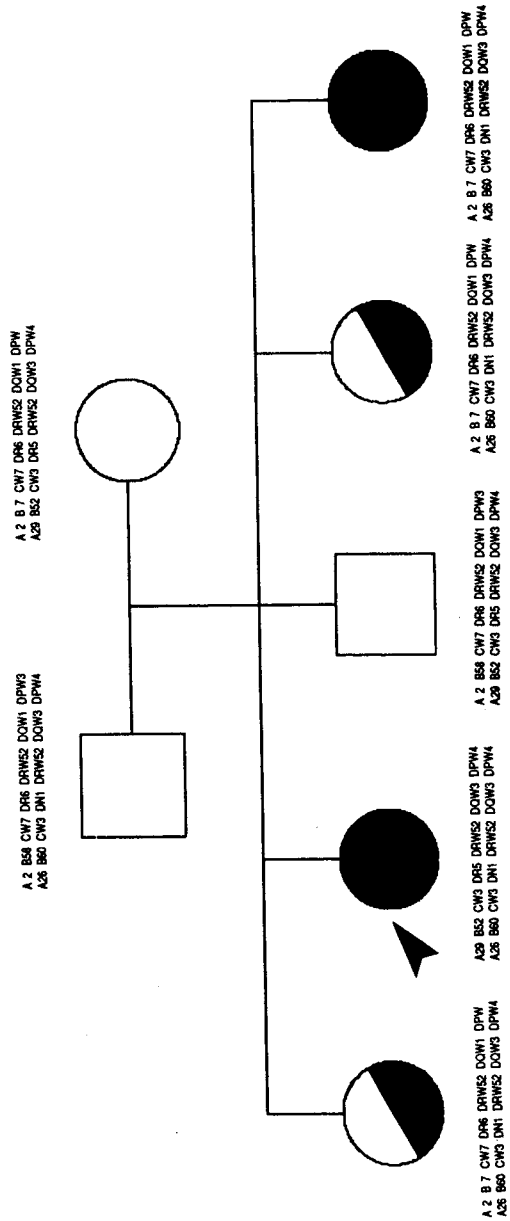


Figure 1. Pedigree and HLA types for family EP033. Note that there has apparently been a crossover in the proband. The entire HLA region appears to have been encompassed by the recombination event. This would indicate that the locus for JME and the EEG traits lay outside the HLA region.

EEGs (Fig. 1). The HLA genotypes of 3 of the sibs are the same. The fourth, who is the proband, had clinical epilepsy but shares only one haplotype in common with the other affected sibs. This family provides evidence that at least one recombination event (i.e., a crossover) must have taken place, assuming inheritance is recessive. There was no evidence of recombination within the HLA region. Thus, any recombination event would have had to occur either outside the HLA region or at least beyond the most outlying tested HLA loci (DP and A). There is as yet little evidence for linkage with GLO1 (glyoxylase) which is on the centromere side of HLA, 14 centimorgans (approximately 1.4×10^7 bases) away, and we had no markers on the other side of the HLA region. The lod score for GLO1 was positive under the assumption of both recessive and dominant inheritance, but did not even reach a value of 1.0.

Especially interesting in these results is that the linkage is with the paroxysmal EEG traits. Not only has the genetic contribution to generalized epilepsy been difficult to demonstrate, the genetics of EEG characteristics has not been clear [Doose and Baier, 1987] and no specific chromosome location of a gene contributing to an abnormal EEG has previously been identified. The linkage reported here may give us a tool to start differentiating forms of generalized epilepsy.

It may seem surprising that the linkage in the case of JME only appeared when the abnormal EEGs were classified as "affected." In general, one imagines that the more specific the definition of the syndrome, the less possibility there is of classifying different syndromes with separate etiologies as one disease. But, in genetic analysis, it is important to designate the phenotypes properly. Misclassification can cause linkage analysis to give misleading results.

This study also demonstrates that linkage analysis can be used to prove that a disease whose genetic component is ambiguous is, in fact, genetic. In our study, we were able to use as a subclinical marker for the presumed disease genotype the abnormal EEG trait seen in clinically unaffected relatives in the linkage analysis. Without that marker, establishing the linkage would have been much more difficult, if not impossible. When we classified the paroxysmal EEGs as "unaffected" the evidence was strongly against linkage at the HLA-BF locus.

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