Genetic mapping of a major susceptibility locus for juvenile myoclonic epilepsy on chromosome 15q

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The epilepsies are a group of disorders characterised by recurrent seizures caused by episodes of abnormal neuronal hyperexcitability involving the brain. Up to 60 million people are affected worldwide and genetic factors may contribute to the aetiology in up to 40% of patients. The most common human genetic epilepsies display a complex pattern of inheritance. These are categorised as idiopathic in the absence of detectable structural or metabolic abnormalities. Juvenile myoclonic epilepsy (JME) is a distinctive and common variety of familial idiopathic generalised epilepsy (IGE) with a prevalence of 0.5–1.0 per 1000 and a ratio of sibling risk to population prevalence ($\lambda_s$) of 42. The molecular genetic basis of these familial idiopathic epilepsies is entirely unknown, but a mutation in the gene $CHRNA4$, encoding the $\alpha_4$ subunit of the neuronal nicotinic acetylcholine receptor (nAChR), was recently identified in a rare Mendelian variety of idiopathic epilepsy, autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) (11). The genes for nAChR subunits have therefore emerged as a class of candidate gene for inherited idiopathic epilepsies.

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$P = 0.0005$. This major locus contributes to genetic susceptibility to JME in a majority of the families studied.

INTRODUCTION

The epilepsies are one of the most common serious neurological disorders with a cumulative incidence of up to 2% by the age of 40 years (1) and up to 60 million people affected worldwide (2,3). Genetic factors may contribute to aetiology in up to 40% of patients (4). Over 100 rare Mendelian disorders include epilepsy as a component of the phenotype, but the most common human genetic epilepsies display a complex pattern of inheritance. These are categorised as idiopathic in the absence of detectable structural or metabolic abnormalities.

Significant progress has recently been made in the mapping and isolation of genes for symptomatic Mendelian epilepsies such as Unverricht–Lundborg disease (5), the neuronal ceroid lipofuscinoses (6–8), Lafora body disease (9) and myoclonic epilepsy with ragged red fibres (10) but the molecular genetic basis of the familial idiopathic generalised epilepsies is entirely unknown. However, a mutation in the gene $CHRNA4$, encoding the $\alpha_4$ subunit of the neuronal nicotinic acetylcholine receptor (nAChR) was recently identified in a rare Mendelian variety of idiopathic epilepsy, autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) (11). The genes for nAChR subunits have therefore emerged as a class of candidate gene for inherited idiopathic epilepsies.
Juvenile myoclonic epilepsy (JME) is a common familial form of idiopathic generalised epilepsy (IGE) characterised by myoclonic jerks on awakening. It is clear that relatives of probands with JME have an increased risk of developing either JME or a related IGE (12). About 7% of the siblings of probands with JME will have epilepsy of which 30% will have JME (12) giving $\lambda_s$ of 0.021/0.0005 = 42. However, the mode of inheritance remains uncertain. Autosomal dominant or recessive, two-locus and polygenic models have all been suggested (13–16).

JME is the only common specific familial idiopathic generalised epilepsy trait to have been subjected to extensive linkage analysis. Previous studies have provided evidence both for and against the existence of a locus designated EJM1 which predisposes to JME or related IGEs on chromosome 6p (17–21). However, it has not proved possible to refine the localisation of EJM1 or to clarify the exact phenotypic trait to which it confers susceptibility (20,22). Nineteen families used in the present study were previously analysed using seven highly polymorphic microsatellite loci on chromosome 6p and no significant evidence in favour of linkage to the clinical trait of JME was obtained for any locus (23).

Thirty-four families, including two or more individuals with clinical JME, have been ascertained in a collaborative effort over a period of 8 years. The hypothesis that genes encoding subunits of nAChRs represent candidate susceptibility loci for JME was tested by undertaking linkage analysis between microsatellite loci spanning the chromosomal regions harbouring these genes and the JME trait in these pedigrees.

**RESULTS**

**Initial screen**

A subset of 19 families ascertained initially was analysed with 22 polymorphic loci spanning chromosomal regions harbouring nAChR subunits genes. A total genetic length of 226 cM representing 6% of the genome was investigated including regions on chromosomes 1p and centromeric 1q, 8p, 15q14, 15q24 and 20q (see Materials and Methods).

Parametric linkage analysis assuming either autosomal dominant (AD) or autosomal recessive (AR) inheritance at 70 and 50% penetrance was carried out. Negative pairwise lod scores were obtained for all loci except $D15S118$ and $D15S128$ in the region of $CHRNA7$ on chromosome 15q14. The maximum positive lod score obtained was 1.86 at $\theta = 0.0$ at $D15S118$ assuming AD inheritance with 50% penetrance.

**Linkage analysis of the $CHRNA7$ region on chromosome 15q14**

A further seven marker loci spanning a 20 cM region around $D15S118$ were analysed in a total of 34 pedigrees (see Materials and Methods). The characteristics of the 34 pedigrees included in the analysis are given in Table 1. The data were analysed using both parametric and non-parametric methodology implemented using the program GENEHUNTER (24).

Multipoint parametric linkage analysis was carried out assuming either autosomal dominant or autosomal recessive inheritance with 50% penetrance and making allowance for locus heterogeneity. A maximum multipoint lod score of 4.42 was obtained at a point 1.7 cM telomeric to $D15S144$ at $\alpha = 0.05$ (Fig. 1a) under the assumption of autosomal recessive inheritance. Multipoint linkage analysis was also carried out using VITESSE (25) which yielded comparable results.

Non-parametric linkage (NPL) analysis using GENEHUNTER is implemented by evaluating the multipoint inheritance pattern in the pedigrees and calculating a scoring function to measure whether affected individuals share alleles identical by descent (IBD) more often than expected under random segregation. No prior inheritance parameters are defined, but the admixture test to detect locus heterogeneity cannot be applied. The statistic produced is called the NPL score, and is referred to as $Z_{\text{all}}$ when sharing in sets of relatives larger than just sib pairs is considered. The significance of the observed NPL score can be calculated exactly (24). A maximum total score of $Z_{\text{all}} = 2.94$, $P = 0.00048$, was obtained at $ACTC$ (Fig. 1b).

GENEHUNTER can also estimate how much of the total genetic information has been extracted and can reconstruct probable haplotypes based on the inheritance vectors. In this study, the densely spaced and highly polymorphic markers provide almost complete information, with the calculated information content (I) ranging from 0.8 to 0.99.

The most likely haplotypes were reconstructed from the inheritance vectors. Direct inspection of the haplotypes revealed that the region of chromosome 15 (from $D15S165$ to $D15S1012$ inclusive) was identical by descent in one or both chromosomes in 25 out of 27 sib pairs. Fourteen shared both parental haplotypes IBD, 11 shared one, and two shared neither (total sharing = 72%). The latter two families are therefore unlinked to this region regardless of the mode of inheritance assumed or exact location within the region of any putative susceptibility locus. Clearly these families contribute to the evidence favouring locus heterogeneity. In all three families with an affected parent, the sibs were IBD for this chromosomal region from the unaffected but not the affected parent. This observation is in favour of a recessive mode of inheritance in these families.

In order to try to detect linkage disequilibrium, the following markers were tested using the extended transmission disequilibrium test (TDT) (26): $D15S144$, $ACTC$, $D15S971$ and $D15S1042$. None of these markers yielded significantly positive results.

**DISCUSSION**

JME is a well-defined and distinctive phenotype which was first described in the last century (27), delineated in some detail 40 years ago (28), and is increasingly recognised and diagnosed. Studies of the incidence of epilepsy in relatives of probands with

### Table 1. Structure of pedigrees used in analysis

<table>
<thead>
<tr>
<th>Pedigree structure</th>
<th>No. pedigrees</th>
<th>No. affected per pedigree</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sibling pairs</td>
<td>23</td>
<td>2</td>
</tr>
<tr>
<td>Sibling pairs with affected parent</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Sibling pairs with affected offspring</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Parent–child pairs</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>1st cousins</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>
JME and twin studies provide strong evidence for genetic contribution to its aetiology but segregation patterns indicate a ‘complex’ pattern of inheritance in most studies. Linkage analysis of diseases with complex inheritance involves a number of difficulties which combine to reduce the power of the method and may preclude the generation of unequivocal results comparable to those obtainable in monogenic diseases. These include uncertainties with regard to the definition of the phenotype, the necessity to make assumptions about the mode of inheritance and penetrance of the trait if parametric methods are used, and an increased possibility that locus heterogeneity underlies the trait particularly if a broad definition of the phenotype is used.

A narrow definition of the trait was used in this study. A diagnosis of clinical JME made according to criteria established by the International League Against Epilepsy (ILAE) was used for the definition of ‘affected’ status. Some authors have
attempted to separate a category of ‘classical’ JME from ‘atypical’ JME, the latter having either pyknoleptic absences or 3 Hz spike and wave discharges on the electroencephalogram (EEG) (20). Some have undertaken linkage analysis using EGG abnormalities in clinically normal relatives as evidence of affectedness (17,29), or using a broad definition of the trait, to include patients with other forms of idiopathic generalised epilepsy as affected (19). A narrow definition of the trait combined with the assumption of low penetrance in parametric analysis represents the most conservative approach. It lessens, but cannot eliminate, the risk of introducing heterogeneity with a broad phenotype and reduces the risk of false negative results arising from the classification of non-penetrant individuals with the disease allele as unaffected.

The true underlying mechanism of inheritance of the JME trait is uncertain. One study in an inbred population provided good evidence for an autosomal recessive mode of inheritance (15), but this may not, of course, be a generalisable observation. Most studies suggest a complex pattern of inheritance. This implies that several loci may interact to confer a genetic susceptibility which may also require non-genetic factors for its expression. It is not possible, however, to distinguish between oligogenic inheritance—in which a few loci of substantial effect interact—and true polygenic inheritance determined by a large number of loci each of small effect. It is also impossible to know in advance the degree of locus heterogeneity which underlies the trait. These difficulties are common to a host of important diseases such as diabetes mellitus, asthma, multiple sclerosis and hypertension. Both parametric and non-parametric analysis of the data was therefore undertaken. Only two models of inheritance were tested—dominant and recessive—and the penetrance was set low so that the relatives of probands with JME suggest that it may predispose to a wide range of seizure disorders.

Two pedigrees containing a sib pair appeared definitely unlinked to this region of chromosome 15. One of these contained an individual with onset of absence seizures at age 6. He was initially diagnosed as having childhood absence epilepsy (CAE). The absences ceased at the age of 11 when he started to have myoclonic jerks. Other members of the family who were excluded from the analysis also had CAE. The other unlinked family showed no characteristics that allowed differentiation from the cohort of JME families.

The appropriate criteria for determining the statistical significance of linkage data derived from the analysis of complex diseases has been much discussed (30). It is generally agreed that thresholds should be adjusted if a whole genome search is undertaken or even planned and if multiple models of inheritance and phenotype are tested, but the exact quantitative effects of such multiple testing of markers or models remain uncertain (31–33). In this study a strong prior hypothesis—the involvement of genes encoding subunits of the nAChR—was tested, in contrast to several recent studies in which random searches of the entire genome were undertaken. Moreover, only a single narrow phenotypic definition was used for determination of affectedness status. Relatives with other varieties of epilepsy or seizure disorder were categorised as unknown.

In the light of these considerations, the present data therefore provide strong evidence in favour of a susceptibility locus for JME in this region which contributes to risk in a majority of, but not all, families. The locus lies in a 15.1 cM region on chromosome 15q based on haplotype sharing. The data do not allow refined localisation of the susceptibility locus but the critical region does encompass CHRNA7 which has been mapped to the interval between D15S144 and ACTC using radiation hybrids (data not shown). This corresponds to the region of the peak lod score. Linkage disequilibrium might be present in polymorphic loci close to the susceptibility locus if a founder effect exists but our attempts to isolate highly polymorphic loci from YACs containing CHRNA7 have not yet been successful. Although many other genes lie within the linked region CHRNA7 remains a highly plausible candidate gene. Present evidence suggests a modulatory role for nAChRs in the brain (34). A transgenic mouse deficient in CHRNA7 has been generated (35) and displays a phenotype characterised by a continuous pattern of generalised hypersynchronous 4–7 Hz sharp wave activity on the EEG similar to that found in patients with JME. Further work is required to examine CHRNA7 for mutations in these patients and to determine the range of epilepsy phenotypes to which this locus may confer susceptibility. The occurrence of a wide variety of seizure types in JME and of less specific epilepsy phenotypes in the relatives of probands with JME suggests that it may predispose to a wide range of seizure disorders.

**MATERIALS AND METHODS**

**Subjects**

Families with a variety of epilepsy phenotypes have been ascertained over the last 8 years, latterly under the auspices of a European Concerted Action on the Genetic Analysis of Epilepsy. Those families containing two or more individuals with JME were selected for inclusion in the study. Individuals were classified as affected if they had clinical JME using diagnostic criteria based on the ILAE classification of epilepsies and epileptic syndromes (36). Criteria for diagnosis were an onset between the ages of 7 and 26 of generalised seizures which must include myoclonic seizures; bilateral, single or repetitive, arrhythmic, irregular myoclonic jerks predominantly in the arms, during which consciousness is not impaired. In addition, absence seizures and generalised tonic–clonic seizures may occur. Neurological and cognitive state is normal. In untreated patients the interictal EEG usually shows episodes of generalised spike and wave or polyspike and wave discharges, but the background activity is otherwise normal. Those with other epilepsy phenotypes, febrile convulsions or a single seizure were classified as unknown. Nineteen families were initially included in the study (23). Continued ascertainment of families resulted in a total of 34 pedigrees being available for analysis. These 34 pedigrees contained 165 individuals of which 73 were classified as affected and five as unknown.

**DNA analysis**

Genomic DNA was extracted from white cells using standard methodology. Individuals from 19 pedigrees were typed using 22 marker loci on chromosomes 1p and centromeric 1q, 8p, 15q14, 15q24 and 20q using fluorescence based semi-automated methods on an ABI 373A. Loci investigated were D1S252, D1S495, D1S506, D1S305, D1S484, D8S504, D8S552, D8S258, D8S283, D8S285, D1S128, D15S165, D15S118, D15S117, D15S302, D15S227, D15S297, D20S120, D20S101 and D20S93. The complete group of 34 pedigrees was then typed using the markers pter–D15S165 - 5.1 cM – D15S144 – 6 cM – ACTC – 0.6 cM –
**Linkage analysis and statistical methods**

Absolute allele sizes were converted to numbered alleles using Genetic Analysis System (GAS) (version 2) created by Alan Young. Allele frequencies were estimated from the sample. For the initial screen of 19 pedigrees, analysis was performed under the assumption of AD inheritance with 70% and 50% penetrance and AR inheritance with 70% and 50% penetrance. A disease gene frequency of 0.00086 was assumed for AD inheritance with 70% (high) penetrance, 0.0012 for AD inheritance with 50% (low) penetrance, 0.04 for AR inheritance with high penetrance and 0.049 for AR inheritance with low penetrance (23). Three liability classes were specified to allow for age-dependent penetrance. Liability class 1 included all individuals under the age of 7 years and corresponded to a penetrance of 0. Liability class 2 included individuals aged between 7 and 26 and corresponded to a penetrance of 0.35 for high penetrance, and 0.25 for low penetrance. Individuals over the age of 26 fell into liability class 3 and were assigned the maximum penetrance of either 70 or 50%. A phenocopy risk of 0.001 was assumed. Two point lod scores were obtained using MLINK and LODSCORE from the LINKAGE package of programs (38). Multipoint parametric and non-parametric linkage analysis was performed using GENEHUNTER (24) under the assumptions of AD and AR inheritance with 50% penetrance. These models of inheritance were selected a priori for a proposed genome search using the a priori frequency of 0.00086 for AD inheritance and 0.0007 for AR inheritance. TDT analysis was performed allowing the risk to vary in order to obtain the maximum lod score (HLOD). Multipoint parametric linkage analysis was also performed using VITESSE (25) under the assumption of AR inheritance with 50% penetrance. TDT analysis was performed for markers D15S971, ACTC, D15S1042 and D15S1042 using the extended transmission disequilibrium test (26).

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**REFERENCES**


