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Evidence for Linkage of Adolescent-Onset Idiopathic Generalized Epilepsies to Chromosome 8—and Genetic Heterogeneity

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Summary

Several loci and candidate genes for epilepsies or epileptic syndromes map or have been suggested to map to chromosome 8. We investigated families with adolescent-onset idiopathic generalized epilepsy (IGE), for linkage to markers spanning chromosome 8. The IGEs that we studied included juvenile myoclonic epilepsy (JME), epilepsy with only generalized tonic-clonic seizures occurring either randomly during the day (random grand mal) or on awakening (awakening grand mal), and juvenile absence epilepsy (JAE). We looked for a gene common to all these IGEs, but we also investigated linkage to specific subforms of IGE. We found evidence for linkage to chromosome 8 in adolescent-onset IGE families in which JME was not present. The maximum multipoint LOD score was 3.24 when family members with IGE or generalized spike-and-waves (SW) were considered affected. The LOD score remained very similar (3.18) when clinically normal family members with SW were not considered to be affected. Families with either pure grand mal epilepsy or absence epilepsy contributed equally to the positive LOD score. The area where the LOD score reaches the maximum encompasses the location of the gene for the β3-subunit of the nicotinic acetylcholine receptor (*CHRNB3*), thus making this gene a possible candidate for these specific forms of adolescent-onset IGE. The data excluded linkage of JME to this region. These results indicate genetic heterogeneity within IGE and provide no evidence, on chromosome 8, for a gene common to all IGEs.

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Introduction

Idiopathic generalized epilepsy (IGE [MIM 600669]) consists of several mostly age-related, clinically distinct syndromes with overlapping symptoms (Commission on Classification and Terminology of the International League against Epilepsy 1989). IGE clusters in families, indicating a genetic basis of the disease (Beck and Janz 1991). The high concordance rate in MZ twins versus DZ twins further suggests that there is an almost exclusively genetic cause of IGE and that nongenetic (environmental) factors play only a minor role (Gedda and Tatarelli 1971; Berkovic et al. 1994). However, how the common forms of IGE are inherited is unknown. The mode of inheritance of IGE is thought to be complex, and the involvement of more than one gene in the expression of IGE has been suggested (Greenberg et al. 1988*a*, 1992). The major complicating factor in the study of the genetics of IGE is genetic heterogeneity. Different genes may cause similar, clinically indistinguishable seizure types. One way of reducing this problem of genetic heterogeneity is careful clinical diagnosis with stringent, well-defined selection criteria imposed at the outset of the study. These clinical differentiation criteria may help to define a clinically homogeneous disease phenotype and thus to identify a more genetically homogeneous study population.

This approach has been productive in juvenile myoclonic epilepsy (JME [MIM 254770]), an IGE subform with adolescent-onset myoclonic seizures. Strong evidence for linkage to the HLA region on chromosome 6p has been found in three independently collected data sets (Greenberg et al. 1988*b*, 1989, 1997; Durner et al. 1991; Weissbecker et al. 1991). Interestingly, Sander et al. (1995) also found evidence for the involvement of this locus on chromosome 6 in families ascertained through a proband with absence epilepsy, but only when an additional family member was affected with JME. A fourth study group found little or no evidence for a JME

locus on chromosome 6 (Whitehouse et al. 1993; Elmslie et al. 1996) but showed linkage to chromosome 15 markers, under the assumption of a recessive mode of inheritance and 65%-linked families (Elmslie et al. 1997). Liu et al. (1996) investigated an extraordinary pedigree with JME from Belize and found linkage to markers centromeric of HLA, raising the possibility of ethnic diversity in JME.

Several genes for epilepsies or epileptic syndromes, as well as genes that might be involved in epileptogenesis (i.e., candidate genes), have been mapped—or suggested to map—to chromosome 8 (fig. 1). Zara et al. (1995) reported suggestive evidence for linkage of IGE to 8q24, the same region in which the gene for benign familial neonatal convulsions (EBN2 [MIM 121201]) (Lewis et al. 1993) has been localized. A defect in a potassium-channel gene has been identified as the cause of EBN2 (Charlier et al. 1998). Evidence for linkage to chromosome 8 has also been reported both in a large pedigree with childhood absence epilepsy (Fong et al. 1998) and in a large family with febrile convulsions (Wallace et al. 1996). A gene locus for a recessively inherited progressive epilepsy with mental retardation (MIM 600143), which, so far, has been found only in a small population in northern Finland, has been assigned to the telomeric end of chromosome 8p (Tahvanainen et al. 1994).

The candidate genes on chromosome 8 that have a conceivable role in epileptogenesis are the genes for a glutamate-binding subunit of an NMDA receptor (GRINA) (Lewis et al. 1996) and for the β3-subunit of the neuronal nicotinic acetylcholine receptor (Koyama et al. 1994). Stargazer and jerky are thought of as mouse models for human epilepsy. In both cases, the human equivalent of the mutated gene maps to chromosome 8 (Noebels et al. 1990; Morita et al. 1998).

Epilepsy genes, like many other gene families, have been found in clusters. On chromosome 20, for example, the locus for autosomal dominant frontal lobe epilepsy (ADNFLE [MIM 600513]) (Steinlein et al. 1995) is located near the locus for benign neonatal familial convulsions (EBN1 [MIM 121200]) (Leppert et al. 1989; Singh et al. 1998) and near an EEG-variant (low-voltage EEG) (Steinlein et al. 1992). Therefore, those areas in which linkage to chromosome 8 has been reported for the aforementioned epilepsies or epileptic syndromes represent viable candidate *regions* for IGE.

We investigated families with adolescent-onset IGE, for linkage to markers spanning the whole length of chromosome 8, including all the markers that have previously been shown to be linked to other epilepsies and epileptic syndromes or candidate genes. We further investigated linkage to specific subforms of IGE and separately analyzed families with JME and other adolescent-onset IGE forms. We found evidence for linkage to chromosomal region 8p11–12, close to the centromere,

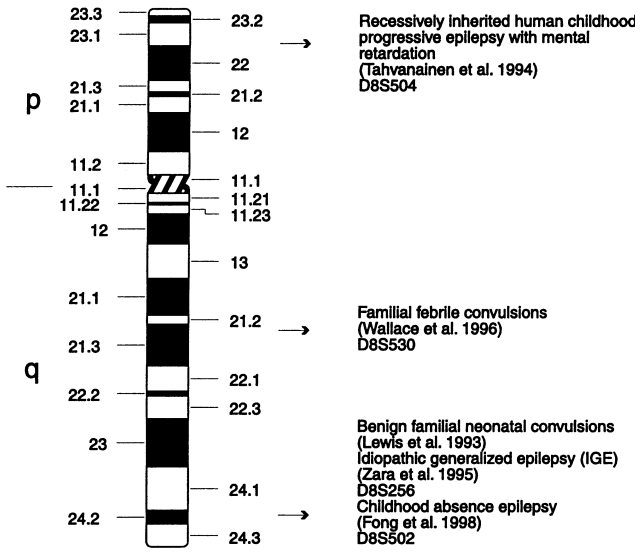

Figure 1 Chromosome 8, with approximate locations of markers for which linkage with several epilepsy syndromes has been reported.

in specific adolescent-onset forms of IGE, which are characterized by the absence of myoclonic jerks. Epilepsy with pure grand mal and absence epilepsy equally contributed to the positive LOD score. The area encompasses the locus for the β3-subunit of the nicotinic acetylcholine receptor, thus making this gene an ideal candidate locus.

Subjects and Methods

Patients

We studied 88 families identified through a proband with adolescent-onset IGE. The diagnosis in probands and affected family members was made according to the revised classification of epilepsy and epileptic syndromes that has been published by the Commission on Classification and Terminology of the International League against Epilepsy (1989). Fifty-two probands had JME, and 32 probands had other forms of adolescent-onset IGE. Of these 32 probands, 20 had pure grand mal epilepsies (i.e., IGE with generalized tonic-clonic seizures as the sole seizure type), and 8 had juvenile absence epilepsy (JAE). Fifteen probands with pure grand mal epilepsy had generalized tonic-clonic seizures randomly occurring during the day (random grand mal [RGM]), and five probands had generalized tonic-clonic seizures occurring predominantly after awakening (awakening grand mal [AGM]). It is important to note that *all* the probands had IGE with seizure onset at 10–20 years of age.

Families

Of the 88 families, 50 (57%) were nuclear families, and 38 (43%) families were multigenerational. Forty-seven family members, in addition to the proband, were affected with IGE. The seizure types seen in these family members included myoclonic jerks (in 15 family members) and generalized tonic-clonic seizures (in 35 family members). Absence seizures were seen in 16 family members. In families of a JME proband, JME occurred in 12 of 25 family members affected with epilepsy. Three families, each ascertained through a proband with IGE with RGM, had a family member affected with JME. Two family members with tonic-clonic seizures had a history of febrile convulsions. One additional family member had a history of febrile convulsions only. A 1-h electroencephalogram (EEG), including hyperventilation and photic stimulation, was performed on 347 family members. Ten family members without either a diagnosis of epilepsy or evidence of seizures showed generalized SW during the recording.

All participating patients and family members gave informed consent. A careful medical and family history was taken from every participating member. Diagnoses were verified by interviews of family members who witnessed the seizures.

Markers

Lymphocytes were isolated from peripheral blood by Ficoll-Paque gradients (Pharmacia Biotech), treated with Epstein-Barr virus (American Tissue Culture Collection), and grown in MEM 1640 media (Glick 1980). Genomic DNA was extracted from those lymphoblastoid cell lines by standard phenol-chloroform methods (Sambrock et al. 1989). Twenty-three markers spanning the length of chromosome 8 were tested (8p tel–D8S504-D8S264-D8S277-D8S550-D8S265-D8S258-D8S282-DD8S535-D8S1758-D8S283-D8S285-D8S260-D8S530-D8S279-D8S273-D8S270-D8S257-D8S281-D8S514-D8S263-D8S284-D8S256-D8S272–8q tel). The markers either were part of the ABI PRISM linkage map (Perkin-Elmer) or were chosen from the Généthon map (Dib et al. 1996). Microsatellite polymorphisms were amplified by PCR in accordance with standard protocols (Weber and May 1989; Ziegle et al. 1992). Markers from the ABI PRISM map were fluorescent labeled, and allele counts were detected with an ABI 310 genetic analyzer. The remaining markers were radiolabeled, and PCR products were separated by standard electrophoresis in Sequagel XR polyacrylamide gels (National Diagnostics) and were visualized by autoradiography. Genotyping was performed without knowledge of the clinical affectedness status.

Linkage Analysis

Linkage analysis was performed by maximum-likelihood methods. We used the program GENEHUNTER (Kruglyak et al. 1996) for multipoint LOD-score analysis. We analyzed the data by assuming a dominant mode of inheritance with 50% penetrance and a recessive mode of inheritance with 70% penetrance. These penetrance values were chosen arbitrarily, according to the recommendations of Hodge et al. (1997). Greenberg (1989) has shown that varying the penetrance values in the analysis can have only limited effect on the maximum LOD score.

Family members were classified under two affectedness models. In affectedness model I (aff1), family members with IGE were considered to be affected. The IGEs in family members comprised JME, epilepsy with generalized tonic-clonic seizures, and absence epilepsy. For affectedness model II (aff2), family members with either IGE or generalized SW were classified as affected. Febrile convulsions were not included in any affectedness model.

We analyzed all the families of probands with IGE ("IGE families") together. We then separated these families into IGE families with JME and IGE families without JME, for the subsequent analysis. Three families were ascertained through a patient with a non-JME form of IGE but had a family member affected with JME. Because of the ambiguous classification and a possible syndrome overlap that would lead to an increase in heterogeneity, we did not consider these families as being within the category of IGE families without JME. We will refer to those IGE families in which JME is not present as "non-JME families."

Results

We were able to exclude a gene locus common to all forms of IGE on chromosome 8, for all models of affected status, assuming either a dominant (fig. 2*a*) or a recessive (fig. 2*b*) mode of inheritance. The multipoint LOD scores ranged between -2 and -13. At D8S256, the marker for which Zara et al. (1995) found evidence for linkage with IGE, the LOD scores were -6.8 (aff1) and -8.7 (aff2), when a recessive mode of inheritance was assumed, and -6.3 (aff1) and -7.2 (aff2), when a dominant mode of inheritance was assumed. In JME families, there was also evidence *against* linkage on chromosome 8, under all affectedness models and transmission models (fig. 3*a* and *b*).

For a recessive mode of inheritance, a LOD score >3 was observed in IGE families without JME, encompassing a broad chromosomal region (fig. 4*b*). This region, from D8S535 to D8S285, peaked at D8S1758, with a multipoint LOD score of 3.24 for aff2 (IGE+SW).

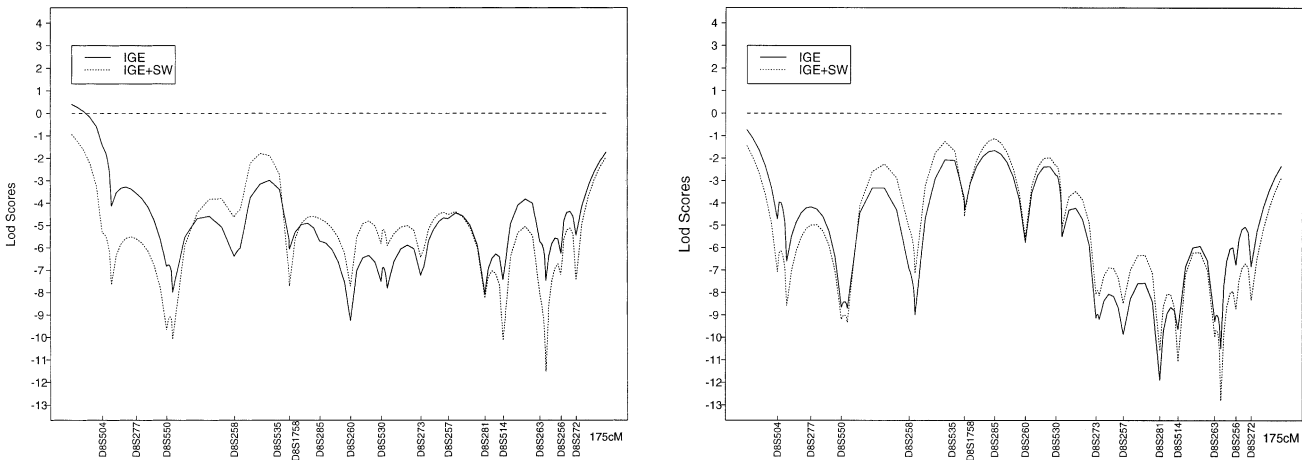

Figure 2 Multipoint LOD scores under the assumption of a dominant (*a*) and a recessive (*b*) mode of inheritance, with chromosome 8 markers in families with IGE.

When clinically normal family members with SW EEGs were not classified as affected, the LOD score remained similar (maximum LOD score 3.18), although the area in which a LOD score >3 occurred was smaller, D8S535–D8S1758. When a dominant mode of inheritance was assumed in the analysis, the LOD score followed approximately the same pattern as was observed in the analysis with a recessive mode of inheritance, but the LOD scores were lower (the maximum LOD scores were 2.1 [for aff1] and 2.4 [for aff2]) and peaked in the same interval (fig. 4*a*).

We then investigated whether a specific subform of non-JME IGE could be identified as contributing to this LOD score of 3.24. Families of probands with RGM epilepsy gave maximum LOD scores of 1.47 (for aff1) and 1.60 (for aff2). In families ascertained through an adolescent-onset absence-epilepsy proband, we found LOD scores of 1.13 (for aff1) and 1.14 (for aff2). AGM families contributed little to this overall LOD score: 0.4 (for aff1) and 0.5 (for aff2). The linkage information in each of the AGM families was very low.

Discussion

Our results suggest that there is a recessively inherited gene on chromosome 8p, close to the centromere, in families with adolescent-onset IGE but without JME. Families with epilepsy with RGM and families with JAE contributed equally to this LOD score. The location of the maximum LOD score is in an area where the gene for the β3-subunit of the nicotinic acetylcholine receptor, *CHRNB3,* has been mapped. The potential role of nicotinic acetylcholine receptors in epileptogenesis has become prominent since it has been shown that a missense mutation in the α4-subunit of the nicotinic acetylcholine receptor can cause ADNFLE in some families with this epilepsy syndrome. Also, Elmslie et al. (1997) have found evidence for linkage of JME to chromosome 15q, an area that encompasses the gene for the α7-subunit of the nicotinic acetylcholine receptor. The nicotinic acetylcholine receptor belongs to the ligand-gated cation-channel family. The neuronal hyperexcitability in epilepsy may be caused by a disturbed calcium permeability (Rathouz et al. 1996). In addition, there is evidence for the involvement of these receptors in neuronal growth/development (Levitt et al. 1997), and mild migration disturbances (i.e., so-called microdysgenesias) have been associated with IGE (Meencke and Janz 1984). Therefore, the *CHRNB3* gene represents a possible candidate gene for adolescent-onset IGEs, which shows linkage to this area on chromosome 8.

The data exclude linkage of JME to this region. Interestingly, three families identified through a non-JME IGE patient but in which a family member was affected with JME also show negative evidence for linkage. This observation suggests that the presence of JME in a family makes that family of the "JME type," even though the family was ascertained through a non-JME proband. This echoes the results reported by Sander et al. (1995), who found evidence for linkage to chromosome 6p in JME families as well as in families of patients with absence epilepsy, but only when they had an additional family member with JME. Linkage to chromosome 6 could be excluded in those absence families in which JME was not present (Sander et al. 1995).

We have shown elsewhere (Greenberg et al. 1995) that epilepsy with RGM does not map to the JME locus (EJM1) on chromosome 6. In that study, families with AGM epilepsy showed suggestive evidence for linkage to the EJM1 locus. The linkage data for AGM families

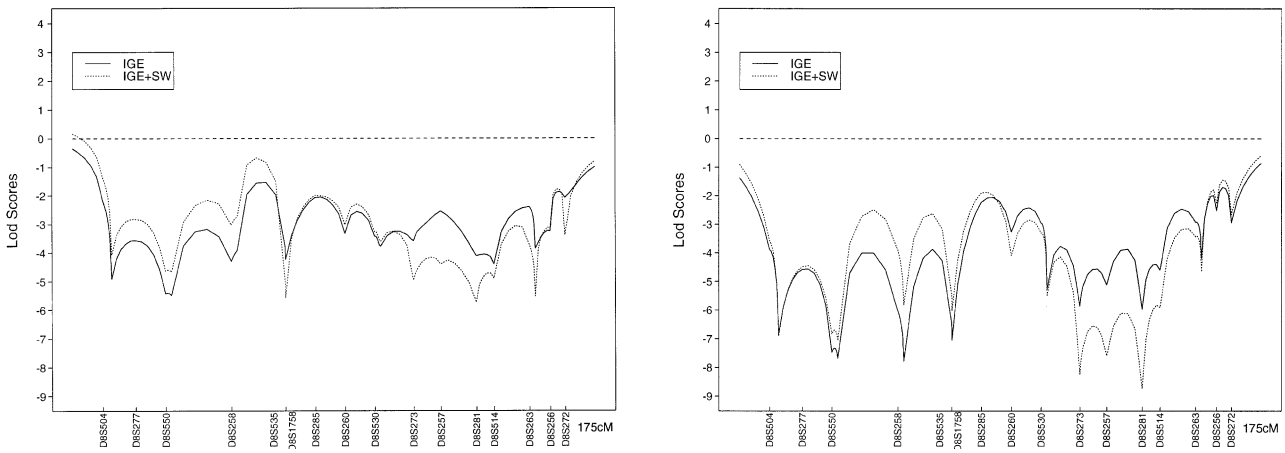

Figure 3 Multipoint LOD scores under the assumption of a dominant (*a*) and a recessive (*b*) mode of inheritance, with chromosome 8 markers in families with JME.

is inconclusive for chromosome 8 because of lack of information, making it impossible to determine whether they behave "JME like."

In families with adolescent-onset IGE that are not JME, the LOD score maximized ~30 cM distant from the locus (D8S530) for which Wallace et al. (1996) reported linkage in a large family with febrile convulsions. This is probably too great a distance to allow us to assume that the two loci identified are identical. However, linkage to the suggested febrile-convulsion loci could not be excluded, because the positive LOD scores in our non-JME families spanned a large area. At D8S530, the LOD scores were still 2.6 (for aff2) and 1.4 (for aff1).

Zara et al. (1995) have reported suggestive evidence for linkage, in IGE families, to markers in the EBN2 region (marker D8S256), in an affected–pedigree-member analysis. When the data were analyzed by multipoint linkage analysis (Kruglyak et al. 1996), the evidence for linkage weakened. This change could be attributed to an increase of linkage information through multipoint analysis. Data from two families that were homozygous for D8S256 became informative for linkage in multipoint analysis and gave strong evidence against linkage (Kruglyak et al. 1996). We have excluded linkage to this marker in the present study's IGE families with LOD scores ranging from -6.3 to -8.7, depending on either the assumed mode of inheritance (dominant and recessive) or whether family members with only SW were included as affected. One difference between our family data and those of Zara et al. (1995) is that the latter investigators classified as affected not only family members with IGE but also those with febrile convulsions. There is some debate as to whether febrile convulsions are a separate disease entity (Nelson and Ellenberg 1981). In our analysis, we chose not to classify those with febrile convulsions as affected. In addition, only a few family members reported having had febrile convulsions. Of those, only one family member had febrile convulsions and no other nonfebrile seizures. Changing the affectedness status of this family member would have made little difference to the overall negative LOD score.

We emphasize that we would have missed the evidence for linkage to D8S535–D8S285 in non-JME families if we had relied only on statistical methods to detect heterogeneity in IGE. When the LOD scores of all families with IGE were also maximized with respect to α (i.e., the proportion of linked families), we found heterogeneity LOD scores between 0.4 and 1.0 at $\alpha = 35\%$–40%. Statistically, these results indicate no significant evidence for heterogeneity. However, because we chose to use clinical criteria to differentiate between different forms of epilepsies, we have been able to show stronger evidence for linkage in a IGE subgroup that is characterized by the lack of juvenile myoclonic jerks.

It is interesting to note that the criterion of myoclonic jerks that is sometimes overlooked or not brought to the attention of the physician plays a decisive role in excluding linkage to chromosome 8, as well as in showing linkage to chromosome 6 in JME families. Careful evaluation, not only of the proband but also of family members, is necessary in all studies of the genetics of epilepsy, because misdiagnosing or not diagnosing JME in family members will increase the amount of heterogeneity in the family data. Information from many families is necessary to disentangle heterogeneity, by available statistical methods. Data collection in IGE, however, is costly and time intensive. The more efficient way to proceed is to minimize heterogeneity, by clinical means at the

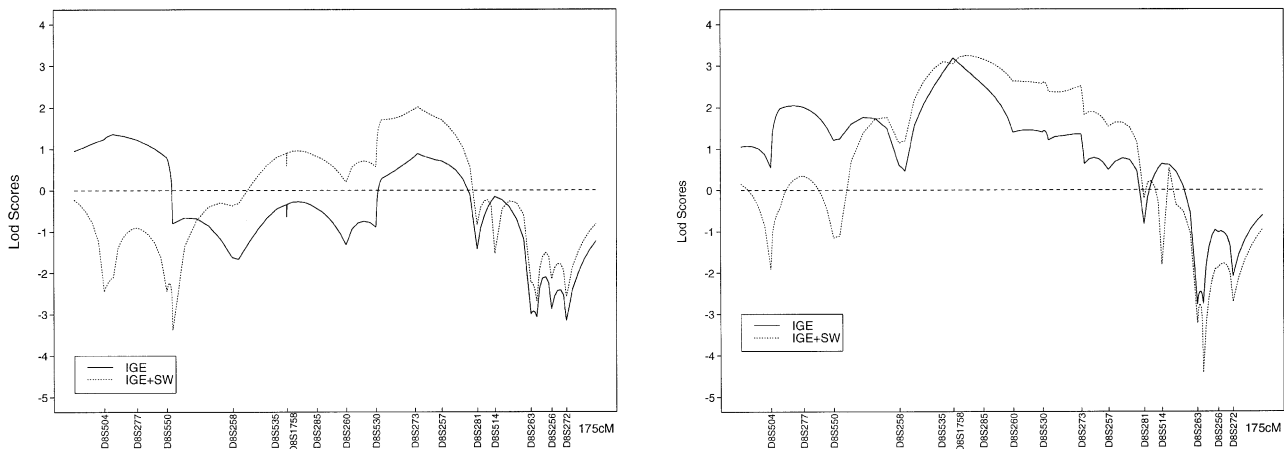

Figure 4 Multipoint LOD scores under the assumption of a dominant (*a*) and a recessive (*b*) mode of inheritance, with chromosome 8 markers in families with non-JME forms of IGE.

outset of the study, so that fewer families will be needed to establish evidence for or against linkage.

We chose LOD-score methods rather than nonparametric methods because of the nature of families with IGE and because LOD-score methods are considered to be more powerful than nonparametric methods (Goldin and Weeks 1993; Durner et al. 1999). The apparent disadvantage of LOD-score methods is that they need the specification of the inheritance model, which is unknown for most of IGEs. However, extensive research has shown that, even for more-complex genetic models (i.e., possible involvement of more than one gene in the etiology of the disease), assuming a simple autosomal dominant or recessive model with reduced penetrance can provide a good approximation when linkage is tested for one gene at a time (i.e., separately) (Greenberg and Hodge 1989; Vieland et al. 1992, 1993; Greenberg et al. 1998). It has been shown that one important factor in LOD-score analysis of complex traits is the assumed mode of inheritance *at the tested locus* and that it is not absolutely necessary to have the overall genetic model of the disease per se correct. In addition, we chose to collect data from families that reflect the population of families with IGE in general. Large pedigrees with many affected family members are the exception, rather than the norm, in IGE, and they raise the suspicion that they are also genetically different from the "garden variety" ones, just because there are so many affected family members. Our data set consists, therefore, mostly of simplex families and of only a few families with more than two affected members, thus making them unsuitable for "model-free" tests, such as affected-sib-pair tests or nonparametric linkage tests (Kruglyak et al. 1996). By using LOD-score methods, we also obtain information from unaffected family members, thereby increasing the overall information content of the family material.

The traditional value for declaring that there is significant evidence for linkage is a LOD score >3. However, we have explored our data and have tested several hypotheses. Therefore, we increase the type I error (i.e., the probability to find false evidence for linkage). We have tested these data with two different genetic transmission models and with two different affectedness models. Hodge et al. (1997) have shown that the increase in type I error due to testing with two dominance models can be compensated by increasing the LOD score cutoff by 0.3 LOD-score units; that is, a LOD score of 3.3, maximized over two dominance models, corresponds to a LOD score of 3 when these data are analyzed under only one mode of inheritance. The effect that testing with two affectedness models has on the significant level is hard to determine, because the two affectedness models (IGE with SW and IGE without SW) are not independent. If the subclinical trait of SW were truly part of this trait, then the analysis considering those subjects as unaffected would classify them as nonpenetrant but still would give positive evidence for linkage (as is the case in our analysis).

We also emphasize that the classification, for analysis, of the families into "JME" and "non-JME" categories was not an ad hoc or post hoc classification. In adolescent-onset IGE there is substantial overlap in seizure types between different syndromes. This overlap can be seen in patients and in family members. For example, 30% of patients with JME have absence seizures in addition to myoclonic jerks, and almost all patients with JME who come to the attention of a physician have generalized tonic-clonic seizures, mostly on AGM (Janz

1989). In families of JME patients, family members have also been found to have JME, but some members had absence epilepsy or pure grand mal epilepsy. Similarly, in families of absence-epilepsy patients, ~20% of family members are affected with JME (Beck and Janz 1991). Several authors have hypothesized that there may be a gene common to all those IGEs and that a second, unique gene will be necessary to determine the specific seizure type (Greenberg et al. 1992; Janz et al. 1992). It was our intention to search for a locus common to all forms of IGE. However, because JME has already been shown to be a genetically distinct form of IGE, the next cogent hypothesis is to remove that known form from the data and reanalyze, which we did.

We observed positive LOD scores covering a broad area, and the peak length was >80 cM. Terwilliger et al. (1997) have shown, both analytically and in simulation studies, that true peaks are, on average, longer than false-positive peaks (i.e., those due to random fluctuations) and that longer peaks are more likely to contain the gene of interest than are shorter peaks. The observation of a long positive peak in our data further strengthens the evidence for a potential gene for some forms of IGE, in this area on chromosome 8.

We have obtained a LOD score of 3.24 in a clinically distinct subgroup of IGE. Our study was able to create strong support for one of our hypotheses—that is, that, among patients with IGE, those without JME in the family are genetically different from those with JME in the family and that there is suggestive evidence of a gene locus for these non-JME IGEs on chromosome 8. However, confirmation with an independent data set will be necessary to support this finding.

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Electronic-Database Information

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

Généthon, http://www.genethon.fr (for chromosome 8 markers)

Online Mendelian Inheritance of Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for ADNFLE [MIM 600513], EBN1 [MIM 121200], EBN2 [MIM 121200], IGE [MIM 600669], JME [MIM 254770], and regressively inherited progressive epilepsy with mental retardation [MIM 600143])

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