

Genome Scan of Idiopathic Generalized Epilepsy: Evidence for Major Susceptibility Gene and Modifying Genes Influencing the Seizure Type

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Idiopathic generalized epilepsy (IGE) is a common, complex disease with an almost exclusively genetic etiology but with variable phenotypes. Clinically, IGE can be divided into different syndromes. Varying lines of evidence point to the involvement of several interacting genes in the etiology of IGE. We performed a genome scan in 91 families ascertained through a proband with adolescent-onset IGE. The IGEs included juvenile myoclonic epilepsy (JME), juvenile absence epilepsy (JAE), and epilepsy with generalized tonic clonic seizures (EGTCS). Our linkage results support an oligogenic model for IGE, with strong evidence for a locus common to most IGEs on chromosome 18 (lod score 4.4/5.2 multipoint/two-point) and other loci that may influence specific seizure phenotypes for different IGEs: a previously identified locus on chromosome 6 for JME (lod score 2.5/4.2), a locus on chromosome 8 influencing non-JME forms of IGE (lod score 3.8/2.5), and, more tentatively, two newly discovered loci for absence seizures on chromosome 5 (lod scores 3.8/2.8 and 3.4/1.9). Our data also suggest that the *genetic* classification of different forms of IGE is likely to cut across the *clinical* classification of these subforms of IGE. We hypothesize that interactions of different combinations of these loci produce the related heterogeneous phenotypes seen in IGE families.

Ann Neurol 2001;49:328–335

Epilepsy's genetic basis was presumed in antiquity, but only recently have new molecular techniques allowed progress in understanding the underlying genetic causes of idiopathic epilepsies. Genes for rare mendelian idiopathic epilepsies have been identified, e.g., benign familial neonatal convulsions (BFNC),¹ generalized epilepsy with febrile seizures plus (GEFS+)^{2,3} or autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE),⁴ all showing the role of ion channels^{2,3,5,6} and acetylcholine receptors⁴ in epileptogenesis. Facilitating these discoveries was the availability of single large kinships with many affected family members and with clear mendelian disease transmission. However, the common forms of idiopathic generalized epilepsy (IGE) have proved much more difficult to study. There is a familial predisposition

in IGE, but the mode of inheritance is more complex, and high-density pedigrees are rare exceptions. Although the methods for identifying genes for simple mendelian diseases are well-known, defining the genetic contribution to genetically complex diseases such as idiopathic generalized epilepsy, multiple sclerosis, schizophrenia, or diabetes (to name a few) have not been fully developed. It is those diseases that pose a challenge to the field of genetics.

Among complex diseases, IGE is especially appropriate to study, because there are indications that IGE is due mostly to genetic factors and that nongenetic (e.g., environmental) factors probably play a limited role in disease etiology,⁷ although the influence of nongenetic factors cannot be excluded. However, the evidence sug-

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Received for publication Apr 5, 2000, and in revised form Aug 11. Accepted for publication Sep 20, 2000.

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gests that more than one gene might be necessary for disease expression. Data from several different studies (twin and family studies and segregation analysis^{8–11}) are compatible with an oligogenic model for IGE, with two or more genes interacting to produce the epilepsy phenotype.

One difficulty in studying the genetics of IGE is the variability of the epilepsy phenotype. In the adolescent-onset IGEs we studied, the patient can present the clinical picture of juvenile myoclonic epilepsy (JME), juvenile absence epilepsy (JAE), or epilepsy with pure generalized tonic clonic seizures (EGTCS). These syndromes show substantial overlap in seizure types. For example, about one third of JME patients also have absence seizures, the dominant seizure type in JAE, and most patients with JME or JAE also have generalized tonic clonic seizures (GTCS). Furthermore, family members of IGE patients are at increased risk for IGE, but the specific IGE seen in family members often differs from the IGE of the proband.¹² It is striking that, when different IGE syndromes are seen in families identified through adolescent-onset IGE, those syndromes are mostly confined to JME, JAE (and to a lesser extent CAE), and EGTCS. The seizure types seen in patients and in family members are almost exclusively either myoclonic jerks, absence seizures, or generalized tonic clonic seizures. The relationship between these different syndromes is unknown, as are the phenotype/genotype correlations. We hypothesized that there might be a gene common to all these IGEs and that other genes are necessary for disease expression as well as for differentiation into specific epilepsy syndromes.

Linkage studies in IGE have focussed primarily on families of JME probands, because JME is clinically easy to distinguish from other IGEs through its identifying characteristic of awakening myoclonic jerks. Our original approach for studying IGE genetics was to ascertain families through carefully diagnosed probands with a clearly defined epilepsy syndrome. This approach, we anticipated, would reduce the amount of expected heterogeneity. Several research groups found evidence for linkage of JME to the HLA region on chromosome 6p.^{13–18} One research group found no statistically significant evidence for linkage to chromosome 6 in their JME families¹⁹ but reported linkage of JME to chromosome 15.²⁰ This linkage to chromosome 15 could not be replicated in two independent samples.^{21,22} Yet another group found linkage of JME to 6p in a single large pedigree but not to the HLA region.²³

Sander et al¹⁶ found no evidence for linkage to the HLA region on chromosome 6p in absence families (i.e., families ascertained through an absence epilepsy patient). However, when another family member in those absence families was affected with JME, evidence for linkage to chromosome 6p emerged. We made a

similar observation for chromosome 8: Families ascertained through a proband with IGE that was not JME showed linkage to chromosome 8.²⁴ JME families showed strong evidence *against* linkage to chromosome 8. However, when another family member in those non-JME families had JME, those non-JME families had negative lod scores on chromosome 8p, just as did the JME families.

This led us to conclude that defining a family solely by the proband's IGE syndrome may be inadequate for genetic purposes; the epilepsy syndromes in the family must be considered in addition. We further reasoned that the *seizure type* might be the critical phenotype, rather than the specific syndrome. We therefore tried using the *seizure type in a family* (as opposed to the epilepsy syndrome of the proband) as the defining phenotype for linkage analysis, with the implicit assumption that loci might interact in various ways to produce the different subforms of IGE. This hypothesis would explain both the observation of different IGEs in family members and that multiple seizure types are seen in some patients. We undertook a genome scan in families ascertained through probands with adolescent-onset IGE. We analyzed the data for all IGE families together but also investigated linkage for subforms of IGE and analyzed families with myoclonic jerks, absence seizures, and grand mal seizures separately. By the approach of stratifying the families by dominant seizure type, we sought to enrich the data for those linkage signals that would point to the involvement of specific genes in particular seizure types.

Families and Methods

Patients and Families

We studied 91 families identified through a proband with IGE.; 53 probands were diagnosed with JME, 10 with JAE, and 21 with epilepsy with GTCS. In 7 probands the GTCS occurred on awakening, and in 14 probands GTCS occurred randomly during the day. Seven probands had IGE, which could not be clearly assigned to the JME, JAE, or EGTCS groups. Forty-five probands with JME also had GTCS, and 15 had absence seizures. GTCS occurred in 3 of the JAE probands. All probands had a seizure onset between 10 and 20 years of age. The diagnosis was made in accordance with the classification of epilepsy and epileptic syndromes established by the International League Against Epilepsy.²⁵ Thirty-eight families were multiplex or multigenerational (42%). Fifty-two family members of three hundred eighty-eight, in addition to the proband, were affected with IGE. The seizure types seen in affected family members were myoclonic jerks (19), absence seizures (20), and GTCS (37). For 337 relatives a 1-hr EEG registration was performed; 10 of 295 family members without a diagnosis of epilepsy showed an epileptiform pattern in the EEG. The study was approved by the institutional review board of the Mount Sinai School of Medicine. All participating patients and family members gave informed consent.

Genotyping

Four hundred seventy-nine family members were typed for the Perkin-Elmer microsatellite panels from the medium-density linkage mapping set (mixture of versions 1 and 2). This set included 375 markers with a genome-wide average 10 cM resolution. On chromosome 6, 20 additional markers and, on chromosome 8, 16 additional markers were typed from the Genethon map.²⁶ Genotyping was performed using an ABI 310 genetic analyzer (PE Applied Biosystems, Foster City, CA), and allele calls were determined with the Genotyper 2.0 software. Genotypes were checked manually and inconsistencies resolved either by retyping or by discarding questionable genotypes. Additional markers were autoradiographically detected. All study personnel were blinded with regard to diagnosis. The marker data were automatically exported into the study database, in which they were integrated with the already existing phenotype information.

Linkage Analysis

We performed a single-locus lod score analysis using LIPED²⁷ and a multipoint analysis with GENEHUNTER.²⁸ Following the recommendation of Hodge et al.,²⁹ we analyzed the data under a recessive and a dominant mode of inheritance with an arbitrary penetrance of 50% except in the analysis for chromosomes 6 and 8. For chromosome 6 we had prior indication of a dominant mode of inheritance with 70% penetrance.^{15,17} For chromosome 8, the prior analysis suggested a recessive mode of inheritance with 70% penetrance.²² Family members were classified under two affectedness models. In model 1, family members with IGE were considered to be affected ($n = 143$), whereas model 2 also included family members without epilepsy but with epileptiform EEG as affected ($n = 153$). Following our initial hypothesis of a gene common to IGE and different genes specific to the subforms of IGE, we analyzed all IGE families together but also looked for linkage in JME and non-JME families as well as in families with absence seizure separately.

We chose lod score methods because of the nature of our family data (42% were multiplex or multigenerational) and because extensive research demonstrated that lod score methods have more power to detect linkage than "model-free" methods, even when the genetic models are complex.³⁰⁻³⁴ It has been demonstrated that a lod score analysis with a simple dominant and recessive model with reduced penetrance can provide a good approximation of the "true" lod score (i.e., a lod score calculated under the correct model), even when the true model was epistatic, heterogeneous, or additive.³⁵⁻³⁷ Usually, linkage in a genome scan of complex diseases is tested at one marker locus at a time. In lod score analysis, the mode of inheritance at that particular locus seems to be the critical assumption and it seems that it is not absolutely necessary to have the overall genetic model of the disease per se correct. For a given disease locus, a person can have only either one or two disease alleles, which is approximated by a dominant or recessive mode of inheritance at that locus. It appears that the action of other genes in an oligogenic model can be approximated fairly well in a single locus analysis by assuming reduced penetrance.³⁸ Another compelling reason for using lod score methods over nonparametric methods was that we expected to find heterogeneity within IGE; thus far,

lod score methods are the only linkage analysis methods that explicitly allow for heterogeneity in the data.

Results

Chromosome 18

Analysis of all IGE families together yielded strong evidence for linkage at only one locus on chromosome 18. The two-point lod score at D18S474 was 5.2 at a male/female recombination fraction of $\theta_{m/f} = 0.1/0.01$ ($Z_{\max} = 4.9$ at $\theta_m = \theta_f = 0.05$). This lod score was calculated under the assumption of a recessive mode of inheritance and included only family members with IGE (model 1) as affected. Both JME and non-JME families gave positive evidence for linkage at that locus. The lod score in JME families was 3.1 at $\theta_{m/f} = 0.1/0.01$ and in non-JME families 2.2 at $\theta_{m/f} = 0.01/0.01$. Among the non-JME families, the information content of the absence families was the highest, contributing the most to this positive lod score. The multipoint lod score in this region was 3.4, but when we allowed for heterogeneity the lod score maximized at 4.5 with $\alpha = 0.65$ (Fig 1). The lod score peaked at D18S474 and the 1-lod interval was approximately 10 cM.

Chromosome 6

For chromosome 6 we were able to reconfirm the previously observed linkage to the HLA region in JME families. The two-point lod score was 4.2 at a low female but high male recombination fraction ($\theta_{m/f} = 0.5/0.01$), and the multipoint heterogeneity lod score was 2.3 at $\alpha = 0.50$ (for further details see Greenberg et al., 2000). These results suggest evidence for heterogeneity and possible maternal inheritance in JME at the chromosome 6 locus. Non-JME families gave evidence *against* linkage to the HLA region (lod scores between -4 and -7). When all the IGE families were taken together, we observed a heterogeneity lod score of 1.4 at $\alpha = 0.35$ at the HLA loci.

Chromosome 8

We previously reported linkage of families with non-JME forms of IGE to chromosome 8p (Durner et al., 1999). The candidate region encompassed the $\beta 3$ subunit of the nicotinic acetylcholine receptor (CHRN3) locus. Typing additional markers in this area increased the evidence for linkage around D8S283 and D8S1758 in those families (multipoint homogeneity lod score = 3.8 assuming a recessive mode of inheritance with 70% penetrance) but yielded strong evidence against the CHRN3 gene as a candidate gene for non-JME forms of IGE (Keddache et al., submitted). Families with JAE and EGTCs contributed equally to this positive lod score for chromosome 8, and there is no statistical evidence for heterogeneity in these families with non-JME forms of IGE. In contrast, JME families

Chromosome 18

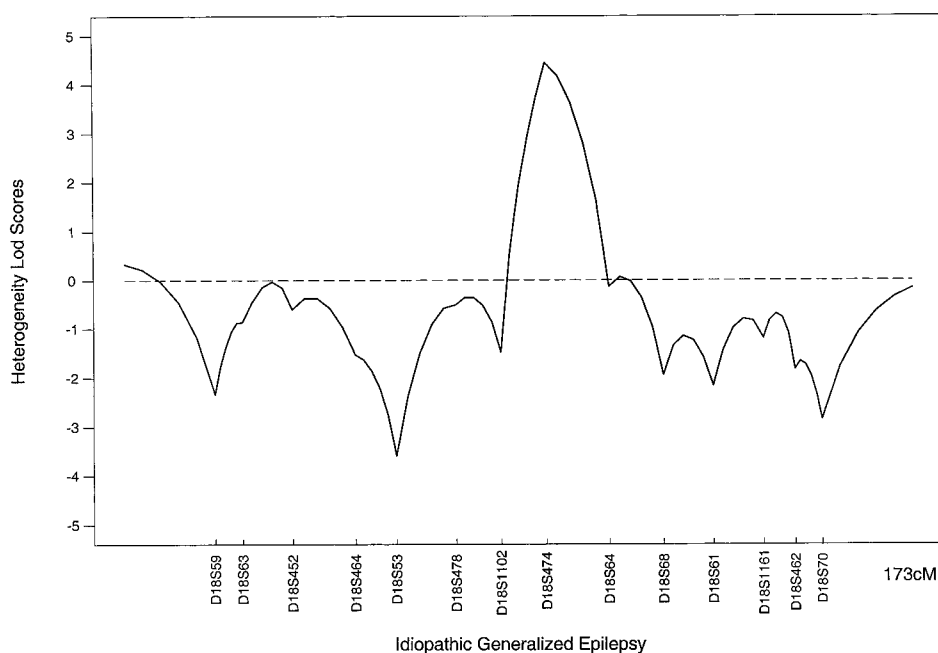


Fig 1. Chromosome 18: multipoint heterogeneity lod scores in families with IGE for affectedness model 1 (IGE) assuming a recessive mode of inheritance with 50% penetrance and $\alpha = 0.65$.

showed evidence *against* linkage on chromosome 8, as did non-JME families with a JME family member.

From the findings on chromosomes 6 and 8, it appears that the presence of myoclonic jerks in the proband or in a family member may serve as a marker for a genetic difference in subgroups of IGE.^{16,24} Reasoning by analogy, we asked the question whether grouping families among whom absence seizures occurred would also produce a genetically consistent group of families, which would allow us to identify a locus influencing absence seizures in the same way in which grouping the JME families allowed us to identify the EJM1 locus.

Chromosome 5

We next grouped all the families with at least one family member with absence seizures, regardless of the epilepsy syndrome, and performed the analysis again. We found two areas on chromosome 5 with lod scores over 3 in this group. At the telomeric end of chromosome 5p, between D5S406 and D5S416, the multipoint homogeneity lod score was 3.8 assuming a recessive mode of inheritance with 50% penetrance (Fig 2). The highest two-point lod score in this area was 2.8 ($\theta_{m/f} = 0.01/0.2$) at D5S406. Assuming a dominant mode of inheritance with 50% penetrance, we observed a multipoint lod score of 3.4 at D5S2027 (Fig 3). However the two-point lod scores in this area were lower (lod score 1.9 at $\theta_{m/f} = 0.01/0.1$), perhaps indicating that this observation is a false-positive result.

Other interesting, but statistically not significant, lod scores were observed on chromosomes 1, 9, 11, and 16 in all IGE families (Fig 4). Multipoint homogeneity lod scores of 2.7 and 2.9 occurred at the telomeric ends of chromosome 9p and 16p, respectively, for model 1 assuming a recessive mode of inheritance. For chromosome 11 a lod score of 2.3 was observed at D11S1345. At the telomeric end of chromosome 1p, a lod score of 2.8 was found for model 2 under the assumption of a dominant mode of inheritance. In families with absence seizures, the lod score between D19S209 and D19S216 on chromosome 19 was 2.7 for model 2 assuming a recessive mode of inheritance.

Type I error (false positives) is an important issue in interpreting the results of a linkage analysis, owing to, in this case, multiple testing. The question arises, how does the number of tests we have performed affect the significance of our findings?

We performed each linkage analysis twice, once assuming dominant and once assuming recessive inheritance. The resulting increase in type I error can be compensated by taking a lod score cut-off of 3.3 instead of the traditional value of 3.²⁹ We also used two models of affectedness. In one, family members with abnormal EEGs were classified as affected, and in one they were classified unaffected. The lod score of ~ 5 at locus D18S474 represents more than 1.5 orders of magnitude greater significance than a lod score of 3.3. The result would still be significant at the 0.0001 level

Chromosome 5

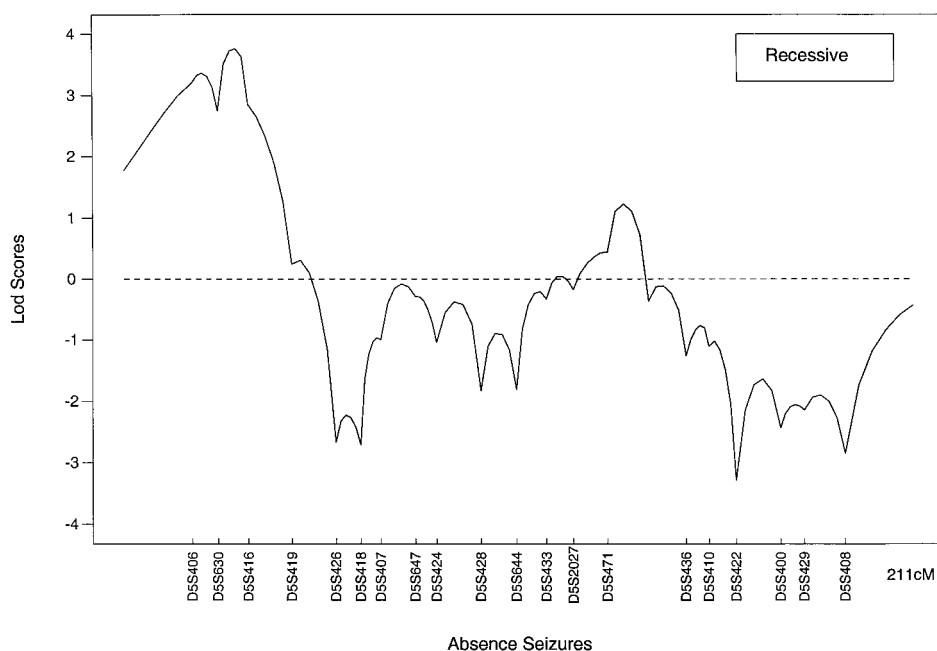


Fig 2. Chromosome 5: multi-point homogeneity lod scores in families with absence seizures for affectedness model 2 (IGE + SW) assuming a recessive mode of inheritance with 50% penetrance.

Chromosome 5

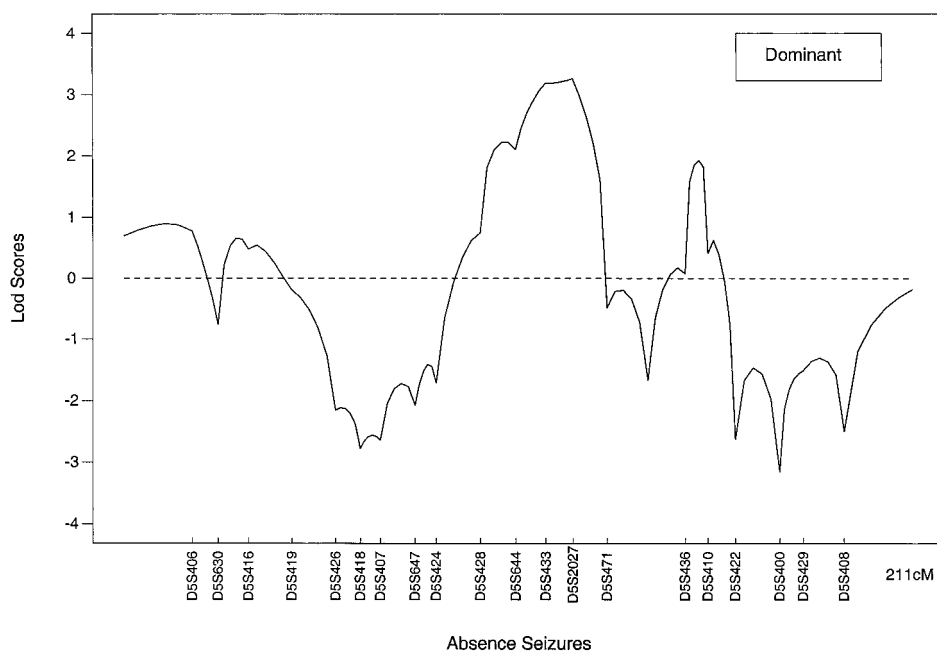
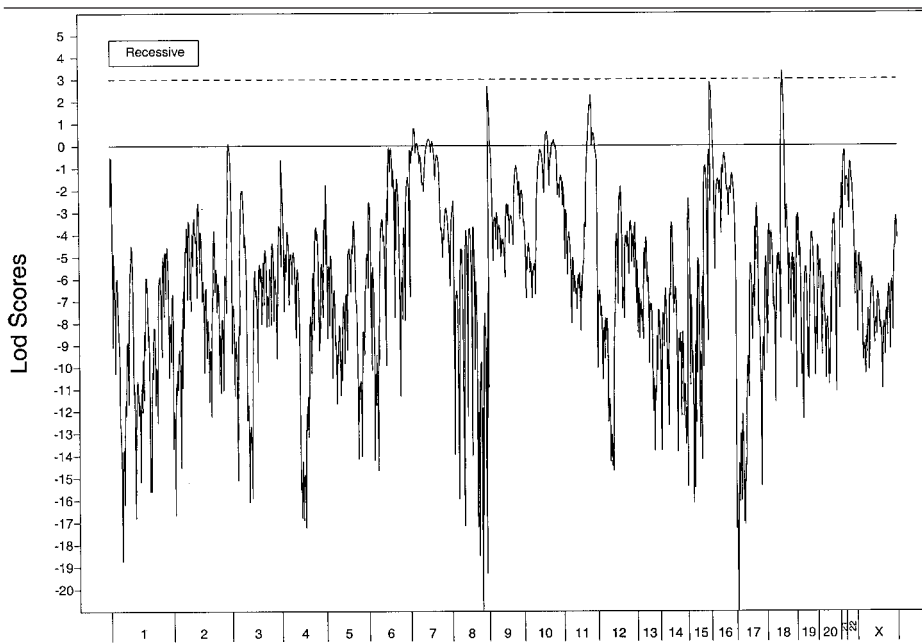


Fig 3. Chromosome 5: multi-point homogeneity lod scores in families with absence seizures for affectedness model 2 (IGE + SW) assuming a dominant mode of inheritance with 50% penetrance.

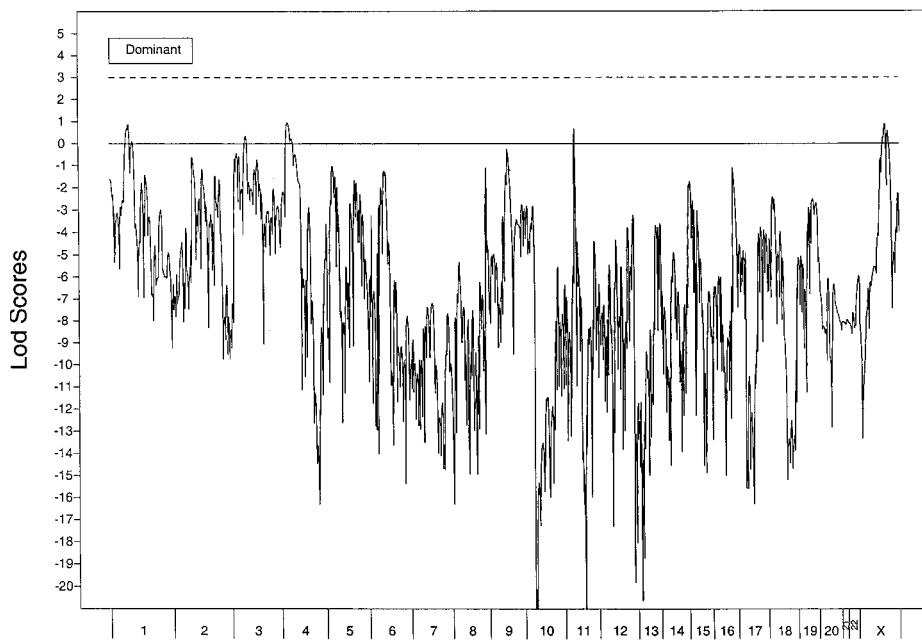
even had we corrected for 200 tests or assumed a χ^2 with 5 df.

The results for chromosome 6, on the other hand, represented confirmation of a previous finding and need no correction for multiple testing.¹⁸ Chromosome

8 was originally singled out, at the beginning of the genome scan, as a chromosome on which epilepsy-related loci had been found. Thus, the significance level of the lod score is higher than had we tested for a location on any of the 23 chromosomes. Another test



A



B

Fig 4. Genome scan in IGE: multipoint homogeneity lod scores in families with IGE for affectedness model 1 (IGE) assuming a recessive mode of inheritance with 50% penetrance (A) and a dominant mode of inheritance with 50% penetrance (B).

was dividing the families into JME and non-JME forms of epilepsy. We had prior evidence that non-JME forms of epilepsy were genetically different from JME,³⁹ and dividing the families into these two groups was not arbitrary.

We do not claim that the results on chromosome 5 for absence represent significant proof of linkage. These results remain a hypothesis to be tested. The

chromosome 5 finding arose out of an attempt to understand why families, even when ascertained through a single IGE syndrome, showed such variable seizure types within the family. We reasoned that, if different loci influenced the presence of different seizure types, then, by grouping all families in which absence occurred, we might uncover evidence for loci influencing the expression of absence. This is precisely what we

had done originally, when we found evidence for linkage to JME, although in that case we did not think of it as grouping families with one seizure type. Rather, we thought of it as collecting families with a specific IGE syndrome. However, in that every patient with JME has to have the seizure type of myoclonic jerks, the effect was the same. This might also imply that the locus on chromosome 6, designated "EJM1," might be not a locus for JME but rather a locus for myoclonic jerks.

Thus, our linkage results are strong evidence for multiple loci, which influence the expression of IGE, even given the moderate number of tests we performed. Because of these multiple hypotheses, we set our cut-off for "interesting" loci at a high lod score of 2.5. Some of those loci, which we did not count as interesting enough to pursue, might still influence the expression of epilepsy.

Discussion

Although IGE shows strong evidence of oligogenetic inheritance, the IGE phenotypes are variable and overlapping. At the beginning of our work, it was unclear how to cope with these overlapping phenotypes. The current work not only represents a report of the localization of IGE-related genes but suggests a new approach in disentangling IGE genetics.

Our results also suggest that multiple genes influence the phenotypes in IGE and also show that the clinical phenotype of an IGE syndrome, which is made up of many different symptoms and characteristics, does not necessarily reflect the underlying genotype(s). Our genome scan found strong evidence for a locus on chromosome 18 possibly common to most, but not all, IGEs. We also found a locus on chromosome 6 in families with myoclonic jerks, a locus on chromosome 8 in families with IGE without myoclonic jerks, and evidence for two loci on chromosome 5 in families with absence seizures. Interestingly, Nakayama et al⁴⁰ found evidence for linkage to the same region on chromosome 5q in a large family with generalized epilepsy plus febrile convulsions as well.

These findings suggest a possible mechanism for how these genes lead to IGE. We propose that the locus on chromosome 18 is a locus that can predispose to epilepsy but that itself may or may not be sufficient to produce seizures. The simultaneous presence of some genotypes at other loci will lead to seizure expression, but the type of seizures produced is strongly influenced, although perhaps not determined, by those other loci present. Thus, one can imagine that, if the chromosome 6 disease genotype is present with the chromosome 18 genotype, then myoclonic jerks will be the seizure type. If the chromosome 5 locus/loci disease genotype is also present, absence seizures will accompany the myoclonic jerks. The chromosome 5 disease

genotype alone, together with the chromosome 18 genotype, will produce an absence phenotype but without the myoclonic jerks. This mechanism would explain why the seizure phenotypes in family members of IGE families can be so variable. It remains likely, given the evidence for heterogeneity, that the chromosome 18 genotype need not always be present and that other loci can interact without it to produce a seizure phenotype. Proving or disproving this hypothesis awaits more data collection.

Our results also suggest that the genetic classification of different forms of IGE is likely to cut across the clinical classification of these subforms of IGE. For example, a patient with the clinical features of absence epilepsy might have the disease form that maps to chromosome 18 and 5, or the patient might have the disease form that is on chromosome 18 and 6, depending on whether the family is JME-like. Other combinations of loci also might result in the absence phenotype. The same might be true for the phenotype of myoclonic jerks; we already have evidence that there is heterogeneity at the chromosome 6 locus in JME families. Unfortunately, there are no clinical differentiation criteria to separate these genotypically heterogeneous phenotypes. Determining the different combinations and interactions of these loci and how they relate to clinical features will be the major challenge in resolving the underlying genetic mechanisms of IGE.

As we stated above, a prerequisite for finding genes involved in IGE was to establish methods that might enable us to detect those genes. We had several a priori hypotheses, which we tested in our data. By doing so, we performed multiple tests. Therefore, the significance levels of our findings will be lowered. However, especially the finding on chromosome 18 will be still highly significant even after correction for multiple testing. More importantly, the observations we made through the testing of our data leads us to propose new strategies and explanations in the genetic research on epilepsy, which are intriguing in that they would integrate several clinical observations.

This study was supported, in part, by National Institutes of Health grants NS27941, DK31775 (D.A.G.), and NS37466 (M.D.) and the Epilepsy Foundation of America (M.D.).

Drs Brannan, Devinsky, Geller, Hauser, Kaufman, Maytal, Novak, Rosenthal, Shanzler, and Walczak each contributed data from one family to the study. We also thank the participating families, who made this work possible.

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