

# A Locus for Generalized Tonic-Clonic Seizure Susceptibility Maps to Chromosome 10q25-q26

Ram S. Puranam, PhD,<sup>1</sup> Satish Jain, MD,<sup>4</sup> Amber M. Kleindienst, BSc,<sup>2</sup> Shilpa Saxena, BSc,<sup>1</sup> Myeong-Kyu Kim, MD,<sup>1</sup> Barbara Kelly Changizi, MD,<sup>1</sup> M. V. Padma, DM,<sup>4</sup> Ian Andrews, MD,<sup>1</sup> Robert C. Elston, PhD,<sup>5,6</sup> Hemant K. Tiwari, PhD,<sup>7,8</sup> and James O. McNamara, MD<sup>1-3</sup>

**Inheritance patterns in twins and multiplex families led us to hypothesize that two loci were segregating in subjects with juvenile myoclonic epilepsy (JME), one predisposing to generalized tonic-clonic seizures (GTCS) and a second to myoclonic seizures. We tested this hypothesis by performing genome-wide scan of a large family (Family 01) and used the results to guide analyses of additional families. A locus was identified in Family 01 that was linked to GTCS (10q25-q26). Model-based multipoint analysis of the 10q25-q26 locus showed a logarithm of odds (LOD) score of 2.85; similar results were obtained with model-free analyses (maximum nonparametric linkage [NPL] of 2.71;  $p = 0.0019$ ). Analyses of the 10q25-q26 locus in 10 additional families assuming heterogeneity revealed evidence for linkage in four families; model-based and model-free analyses showed a heterogeneity LOD (HLOD) of 2.01 ( $\alpha = 0.41$ ) and maximum NPL of 2.56 ( $p = 0.0027$ ), respectively, when all subjects with GTCS were designated to be affected. Combined analyses of all 11 families showed an HLOD of 4.04 ( $\alpha = 0.51$ ) and maximum NPL score of 4.20 ( $p = 0.000065$ ). Fine mapping of the locus defined an interval of 4.45Mb. These findings identify a novel locus for GTCS on 10q25-q26 and support the idea that distinct loci underlie distinct seizure types within an epilepsy syndrome such as JME.**

Ann Neurol 2005;58:449–458

Epilepsy, characterized by recurrent seizures, is a common and complicated group of disorders. The genetic cause of multiple, rare Mendelian forms of human epilepsy have been identified in the past decade,<sup>1-4</sup> and genes potentially conferring susceptibility to some non-Mendelian forms have been detected.<sup>5,6</sup> This progress notwithstanding, the genetic causes of forms of human epilepsy commonly seen in the clinic are incompletely understood.

The most common category of epilepsy with genetic determinants is the idiopathic generalized epilepsies (IGEs).<sup>7</sup> The IGEs include multiple syndromes such as juvenile myoclonic epilepsy (JME), childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), and generalized tonic-clonic epilepsy (GTCE). These syndromes represent complex genetic disorders in that a genetic determinant is present, yet Mendelian segregation is not evident in most families. Evidence of a genetic determinant includes concordance rates of 0.65

and 0.24 in monozygotic and dizygotic twins, respectively.<sup>8,9</sup> In addition, the recurrence risk to first-degree relatives of subjects with IGE is approximately 14-fold that of the general population. This approximation is based on the observation that 5.6% of first-degree relatives of subjects with IGE syndromes exhibit an IGE<sup>10,11</sup>; in contrast, the occurrence of IGE in the general population is 0.4% based on the cumulative incidence of epilepsy of 1%<sup>12</sup> and the estimated occurrence of IGE in 40% of patients with epilepsy.<sup>7</sup>

We studied JME, in particular, because it is a common form of IGE and exhibits a distinctive phenotype which is readily ascertained. JME typically begins early in the second decade of life in an otherwise normal subject and persists throughout the individual's life. It is characterized by myoclonic and generalized tonic-clonic seizures (GTCS). In addition, some subjects exhibit brief absence seizures, the detection of which is more difficult. Inheritance patterns in twins<sup>8,9</sup> and

From the Departments of <sup>1</sup>Medicine (Neurology), <sup>2</sup>Neurobiology, and <sup>3</sup>Pharmacology and Molecular Cancer Biology, Duke University, Durham, NC; <sup>4</sup>Neuroscience Center, All India Institute of Medical Sciences, New Delhi, India; Departments of <sup>5</sup>Epidemiology and <sup>6</sup>Biostatistics, Case Western Reserve University, Cleveland, OH; and Departments of <sup>7</sup>Biostatistics and <sup>8</sup>Pathology, University of Alabama, Birmingham, AL.

Received Feb 28, 2005, and in revised form Jun 27. Accepted for publication Jun 27, 2005.

Published online Aug 29, 2005, in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ana.20598

Address correspondence to Dr McNamara, Department of Neurobiology, 401 Bryan Research Building, Research Drive, Box 3676, Duke University Medical Center Durham, NC 27710.  
E-mail: jmc@neuro.duke.edu

multiplex families<sup>13</sup> led us to hypothesize that two loci were segregating in subjects with JME, one predisposing to GTCS and a second to myoclonic seizures. Among dizygotic twins in which the proband exhibits JME, the subset concordant for epilepsy most commonly exhibits GTCS, not JME itself.<sup>8,9</sup> Likewise, analysis of subtypes of IGE syndromes in relatives of probands with JME shows that affected first- and second-degree relatives are almost equally likely to exhibit either JME (ie, myoclonic seizures plus GTCS) or GTCS alone.<sup>13,14</sup> One explanation for these inheritance patterns is that one gene confers susceptibility to GTCS and a second to myoclonic seizures. The idea of genes conferring susceptibility to a specific seizure type found support from work by Durner and colleagues<sup>15</sup> and Winawer and colleagues.<sup>16</sup> For example, increased concordance of myoclonic or absence seizures among families with IGE with two or more affected subjects was consistent with the idea of seizure-specific genes.<sup>16</sup>

To test the hypothesis that distinct loci segregated with myoclonic seizures versus GTCS, we collected JME proband families from a single ethnic group providing a more homogenous group in which to detect linkage. Importantly, affection status was stratified to permit testing our hypothesis. The initial step was to conduct a genome-wide survey of the largest family of 25 subjects which included 9 affected members in 3 generations. The results from the initial family guided analysis of 10 additional families. The results identify a novel locus for GTCS on 10q25-q26 and are consistent with the hypothesis that distinct loci underlie susceptibility to distinct seizure types in the syndrome of JME.

## Subjects and Methods

### Families

Eleven probands exhibiting JME and their families visiting the All India Institute of Medical Sciences (AIIMS), New Delhi, India, were identified and invited to participate in a study of genetic causes of JME. All probands and family members from whom blood samples were obtained were interviewed for history of seizures and any other neurological disorders and underwent a detailed neurological examination by one of us (S.J.). Scalp electroencephalograms of duration

approximating 20 minutes' duration were performed in all members of Family 01, and most but not all members of the 10 additional families. Blinded analyses of scalp electroencephalograms (EEGs) by J.O.M. disclosed no abnormalities among members of Family 01, and thus inclusion of EEG results provided no further information beyond that obtained from clinical history. Together with the fact that EEGs could not be performed in all members of the 10 additional families, EEG data were not included in phenotypic classification. The diagnosis of JME was based on unequivocal clinical or historical evidence of bilateral myoclonic jerks and GTCS beginning early in second decade with or without absence seizures. Patients with evidence of neurological or intellectual deficit or with evidence of some definable cause of seizures (eg, alcohol withdrawal, substance abuse, brain hypoxia, meningoencephalitis, and degenerative disease) were excluded.

To assure accuracy and consistency of phenotyping, epileptologists at both the AIIMS and Duke University first independently evaluated the clinical data, and the diagnosis was based on joint evaluation of the data. A phenotype was established using the classification presented in Table 1. The institutional review board of both the AIIMS and Duke University approved this study. Informed consent was obtained from all participants.

### Isolation of Human Genomic DNA and Genome Scan

Genomic DNA was isolated using Puregene Kits (Gentra Systems, Minneapolis, MN) from peripheral blood. A genome-wide survey was conducted using the 25 subjects of Family 01 (Fig 1) using 414 markers on 22 autosomes with an average intermarker interval of 10cM. Markers from Research Genetics were used in the initial survey using radioactive methods.

Additional genotyping was performed using primers conjugated with fluorescent dyes from Applied Biosystems (ABI prism linkage mapping set v2.5 HD5). Amplification of genomic DNA and pooling of the polymerase chain reaction products was conducted as per the Applied Biosystems linkage mapping set v2.5 HD5 protocols. The multiplexed panels were resolved on an ABI 310 capillary machine (Applied Biosystems). A Centre d'Etude Polymorphisme Humain (CEPH) control (1347-02) was included in each run. Data analysis and allele calling were performed according to the protocols and programs provided by the ABI 310 Genetic Analyzer package.

Table 1. Phenotype Classification of Individuals in Juvenile Myoclonic Epilepsy Proband Families

1.0	Myoclonic seizures plus tonic-clonic seizures
1.5	Myoclonic seizures alone
2.0	Multiple generalized tonic-clonic seizures, onset up to and including 29 years old
3.0	Single generalized tonic-clonic seizure, up to and including 29 years old
4.0	Three or fewer febrile seizures
5.0	Single or multiple generalized tonic-clonic seizures, age 30–59 years included
6.0	Partial seizures with secondary generalized tonic-clonic seizures
7.0	Incomplete data or patient not examined but history of seizure
8.0	Unaffected
9.0	Unknown

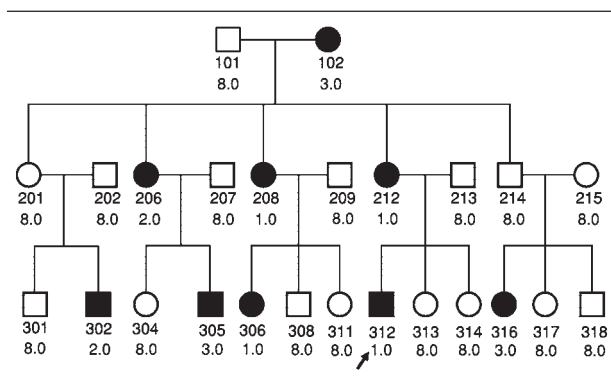


Fig 1. Family 01. The three-generation, 25-member family with the proband indicated by the arrow is presented with the subject identification number and the phenotype of the subjects (see Table 1) shown at the bottom. According to affection status classification (see Table 2), affection status A designates Subjects 208, 212, 306, and 312 as affected and all others as unaffected. Affection status B designates 102, 206, 302, 305, 316 and 208, 212, 306, 312 as affected and all others as unaffected. The numbers of unaffected subjects in affection status A and B are 21 and 16, respectively.

### Fine Mapping

Fine mapping using single-nucleotide polymorphisms (SNPs) was performed to examine the 1.95Mb intervals spanning a putative recombination between markers D10S1269 and D10S1776 in affected Subject 01-305. SNP genotyping was performed using template-directed primer extension and detection by fluorescence polarization<sup>17</sup> using Victor<sup>2</sup> (Perkin-Elmer, Boston, MA) using kits were purchased from Perkin-Elmer.

### Statistical Methods

Error checking and relationship testing were assessed as follows. Inconsistencies in the segregation of genotypes within the families tested were examined with MARKERINFO (S.A.G.E.) and SIMWALK2.89. MARKERINFO detects any Mendelian inconsistencies. SIMWALK2.89<sup>18</sup> was used to identify double crossovers in haplotypes. Mendelian and genotyping inconsistencies at any marker were resolved as much as possible by retyping. Markers resulting in ambiguous allele data were replaced by nearby polymorphic markers. The marker data were checked for any significant departures from Hardy-Weinberg equilibrium proportions. Allele frequencies for each marker were estimated by maximum likelihood using the S.A.G.E. program *FREQ*.

A genome-wide screen was first performed for Family 01 using the model-based linkage analysis program LODLINK that calculates logarithm of odds (LOD) score values for two-point linkage between a trait and each marker. The LODLINK module of S.A.G.E. programs uses the genotype/phase elimination algorithms proposed by Lange and Boehnke<sup>19</sup> and Lange and Goradia<sup>20</sup> to perform fast linkage calculations. The multipoint model-based and model-free allele sharing linkage analyses were performed using GENEHUNTER.<sup>21</sup> The two-point LOD scores and heterogeneity LOD (HLOD) scores were calculated for a dominant model with 65% penetrance. Model-free allele sharing was assessed

with the nonparametric linkage (NPL) statistic, based on estimated allele sharing for all affected relative pairs in the data set. SIMWALK2.89, a statistical genetics computer application using Markov Chain Monte Carlo and simulated annealing algorithms were used to perform multipoint and haplotype analysis.

### Results

A genome-wide survey was conducted for Family 01 (Fig 1) after establishing a phenotype classification (Table 1) and stratification of affection status as a function of distinct seizure type (Table 2). Two-point LOD score analysis was performed to test the hypothesis that two loci were segregating in subjects with JME, one with myoclonic seizures and a second with GTCS. To test this hypothesis, one definition of affection status (see Table 2, status A) required the presence of myoclonic seizures which included either phenotype 1.0 (myoclonic + GTCS) or 1.5 (myoclonic jerks alone). A second definition of affection status (see Table 2, status B) required the presence of GTCS which included phenotypes 1.0, 2.0, and 3.0 (see Table 1) and excluded other phenotypes. The initial model assumed an autosomal dominant (AD) pattern of inheritance, a penetrance of 0.65, no sporadic cases, and a disease allele frequency of 0.0025. Similar results were obtained from analyses assuming either 1% or no sporadic cases (data not shown). The AD pattern was selected because analysis of the affected status of Family 01 suggested an AD pattern. Autosomal recessive models were examined but lack of linkage led to use of the AD model.

Several results were notable. Negative two-point LOD scores were obtained with the majority (>90%) of markers demonstrating inability to establish linkage with the phenotype of myoclonic seizure or GTCS. Two-point LOD scores exceeding 2.0 were detected at only two sites in the entire survey, chromosome 16 and chromosome 10 for affection status A and B, respectively. Moreover, at each of these putative loci two-point LOD scores exceeding 2.0 were detected with adjacent markers (Table 3), and these loci were the focus of the work in this article. The chromosome 16 locus segregating with myoclonic seizures (affection sta-

Table 2. Affection Status Stratification as a Function of Distinct Seizure Types

Affection Status	Phenotypes <sup>a</sup>
A	1.0, 1.5
B	1.0, 2.0, 3.0
C	1.0, 2.0, 3.0, 5.0, 6.0.

<sup>a</sup>Phenotype definitions are as described in Table 1. A single individual with phenotype 4.0 (three or fewer febrile seizures) from Family 04 was considered as unaffected for all affection statuses in this study.

tus A) in Family 01 was examined with additional markers at intervals approximating 2Mb flanking markers D16S397 and D16S3095 (see Table 3). This showed a multipoint LOD score of 2.02 (data not shown). Haplotype analysis showed a chromosomal segment approximating 22Mb that was derived from the unaffected grandfather (01-101) and was transmitted only to Subjects 01-208, 01-212, 01-306, and 01-312, subjects all exhibiting the phenotype 1.0 (see Fig 1) associated with myoclonic seizures. Importantly, a negative multipoint LOD score for affection status B ( $-3.16$ ) was obtained for the chromosome 16 locus, demonstrating that this locus does not segregate with GTCS (see Table 3).

Subsequent studies centered on affection status B because of the greater severity of GTCS compared with myoclonic seizures. In addition, greater numbers of affected subjects with GTCS provided greater statistical power. Multipoint analysis was performed on chromosome 10 using 15 markers (shown in boldface in Fig 3) flanking D10S468 spanning 25.68Mb (28cM) using the same model as for the two-point linkage analyses. This showed a maximum multipoint LOD score of 2.85 with affection status B (Fig 2A). The validity of these findings was strengthened by model-free analyses in which linkage is sought with alleles that are shared by affected subjects using Genehunter 2.1.<sup>21</sup> This showed a peak nonparametric LOD score of 2.7 ( $p = 0.0019$ , see Fig 2B) with affection status B. The location of the peak interval was similar for the model-dependent and model-free analyses (see Fig 2A, B, respectively). In contrast, evidence against linkage to affection status A was evident from model-dependent (see Fig 2A) and model-free analysis (see Fig 2B). Thus, just as the chromosome 16 locus is specific to myoclonic seizures (affection status A), the chromosome 10 locus is specific to GTCS (affection status B).

To define the critical interval in Family 01, a total of 32 markers, including the 15 markers presented in Figure 2, were used to genotype all members of 01, and the data were analyzed with Simwalk 2.89 (Fig 3). A segment of the chromosome inherited from Subject 01-102 segregates with affection status B in all affected subjects. Centromeric recombination in Subject 01-

305 between markers D10S1269 and D10S1776 and telomeric recombination in Subject 01-208 between markers D10S221 and D10S1425 defined a locus of 4.82Mb (4.87cM). This locus is telomeric to LGI1 previously implicated in a form of partial epilepsy.<sup>22</sup> The recombination event within the 1.95Mb interval between markers D10S1269 and D10S1776 in Subject 01-305 was fine-mapped by SNP-based genotyping and was found to be between SNPs rs1362943 and rs1570272 (data not shown). The interval between SNP rs1362943 and marker D10S1425, a distance of 4.45Mb, defined the critical interval of the GTCS locus.

We sought to determine whether the chromosome 10 locus segregated in any of the additional JME proband families recruited from a similar geographical locale (Families 02–11). These 10 families included 53 normal and 41 affected subjects. Among these 10 families, the numbers of affected subjects with a given phenotype (see Table 1) are specified in parentheses after the phenotype: 1.0 (17), 1.5 (2), 2.0 (12), 3.0 (5), 4.0 (1), 5.0 (1), and 6.0 (3). In contrast with Family 01, in which all GTCS were generalized from onset and began in the first three decades (phenotypes 1.0, 2.0, and 3.0), three subjects in these families (02-305 [Fig 4A] and 07-417 and 09-410 [Fig 4C]) exhibited GTCS that were partial in onset and thus secondarily generalized (phenotype 6.0). A fourth subject (03-207, see Fig 4B) exhibited a GTCS in the fourth decade (phenotype 5.0). To designate all subjects with these forms of GTCS as affected, we established an additional affection status (C) that included phenotypes 5.0 and 6.0. That is, affection status C designated these four subjects as affected in contrast with affection status B in which they were unaffected. The 15 markers used for the multipoint analysis for Family 01 (see Fig 2) were genotyped in all members of the 10 additional families and linkage under the assumption of genetic heterogeneity<sup>1,23,24</sup> was sought using the program Genehunter 2.1.<sup>21</sup> Analysis of the 10 families tested independently of the initial family revealed a heterogeneity LOD score (HLOD) of 2.01 and  $\alpha$  value (ie, proportion of linked families) of 0.41 with affection status C, suggesting that linkage could be established in 4 of the additional 10 families (Families 02, 03, 09, and 11). Importantly, designating the four subjects exhibiting the phenotypes 5.0 and 6.0 as unaffected with respect to affection status B led to a HLOD of 0.37 and  $\alpha$  value of 0.28. In addition, model-free analyses of these additional 10 families were performed. The nonparametric LOD score for affection status C was 2.56 ( $p = 0.0027$ ). Similar analyses using affection status B showed a LOD score of 1.74 within this interval ( $p = 0.02$ ; data not shown).

Additional analyses were performed in which all 11 families (01-11) were included using both model-based

Table 3. Two-Point Logarithm of Odds Scores

Marker	Genetic Position	Affected Status A	Affected Status B
D10S254	10q25.1	-1.3	2.29
D10S468	10q25.3	-1.63	3.14
D10S1683	10q25.3	-2.09	2.68
D10S187	10q25.3	-2.09	2.68
D16S397	16q22.1	2.01	-3.16
D16S3095	16q22.1	2.01	-4.55

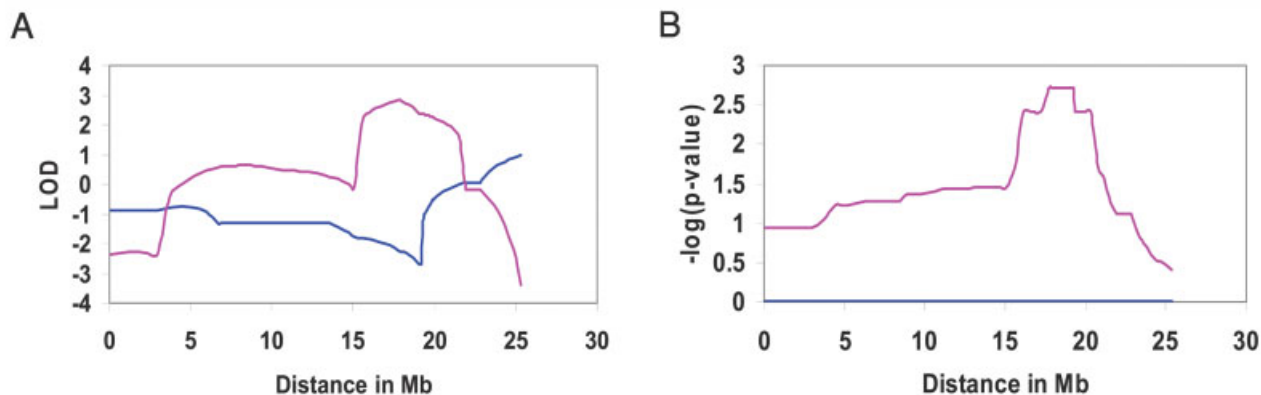


Fig 2. (A) Multipoint logarithm of odds (LOD) scores under affection status A and B for Family 01. The multipoint LOD scores were calculated using Genehunter 2.1 and under the model of autosomal dominant with 0.65 penetrance and disease allele frequency of 0.0025. Because of the limitation of the multipoint model-based linkage analysis programs with respect to the size of the family that can be analyzed, Family 01 was split for these analyses to accommodate  $2n-f$  to be equal or less than 18. (B) Nonparametric  $-\log(p)$  value distribution of Family 01. Model-free allele sharing was assessed with the nonparametric linkage (NPL) statistic, based on estimated allele sharing for all affected relative pairs in the data set and the nonparametric  $-\log(p)$  values were estimated using Genehunter2.1. The maximum multipoint LOD score for affection B was 2.85. The corresponding value for the affection status A at the same position was  $-2.23$ . The maximum NPL scores for affection status B was found to be 2.71 ( $p = 0.0019$ ). A NPL score of 0 was obtained for the affection status A. Affection status A and status B are represented by blue and magenta lines, respectively. The 15 markers used for genotyping are specified in boldface in the left margin of Figure 3.

and model-free analyses. Testing for linkage to affection status C in the 11 families in the presence of heterogeneity showed a HLOD of 4.04 and  $\alpha$  value of 0.51, suggesting that linkage could be established in 5 of the 11 families (Fig 5A). In contrast, affection status B showed a HLOD of 2.26 and  $\alpha$  value of 0.45. Model-free analyses for affection status C showed a nonparametric LOD score of 4.20 ( $p = 0.000065$ ). Similar analyses for linkage to affection status B showed a nonparametric LOD score of 3.62 ( $p = 0.00024$ ). The values for affection status A were not significant (see Fig 5A, B, blue line). Taken together, these data support the presence of a locus on chromosome 10 harboring a gene for GTCS in five families (Families 01, 02, 03, 09, and 11), detected by both model-based and model-free analyses. The critical interval of this locus was determined to be 4.45Mb as defined from the recombination events in Family 01 alone, because the recombination events in the additional linked families were outside this region (see Fig 4A–C, data not shown for Family 11).

The sharp contrast in linkage results obtained with affection status B versus C led us to query how inclusion of these four additional subjects with GTCS might have accounted for the difference. As noted above, in contrast with affection status B, affection status C permits designating any subject with GTCS as affected by virtue of including phenotypes 5.0 and 6.0 (see Table 2). The multipoint LOD scores and haplotypes of the four families to which the subjects with phenotypes 5.0 and 6.0 belonged were examined to elucidate the contribution of these subjects. Three of

the four subjects included in affection status C belonged to the linked families (02, 03, and 09, see Fig 4–C) and the fourth belonged to an unlinked family (07). Designation of Subjects 02-305 and 09-410 with secondarily GTCS as affected (status C) increased the multipoint LOD score from 0.83 and 0.24 for affection status B, to 1.41 and 1.67 for status C in Families 02 and 09, respectively. Designation of 03-207 with GTCS in the fourth decade as affected resulted in minimal effect on the LOD score (0.37–0.39 for status B and C, respectively). Interestingly, evidence against linkage remained strong for Family 07 regardless of whether 07-417 with secondarily GTCS was designated as unaffected (status B, LOD  $-3.5$ ) or affected (status C, LOD  $-2.4$ ). The three subjects (02-305, 03-207, and 09-410) from the linked families shared the same haplotype as the other affected subjects in their families (see Fig 4A–C). These results raise the interesting possibility that the chromosome 10 locus confers susceptibility to GTCS whether generalized from the outset or secondarily generalized.

## Discussion

We hypothesized that JME is caused by the simultaneous inheritance of two susceptibility genes conferring susceptibility to distinct seizure types. We tested this hypothesis by performing a genome-wide scan of a large family (Family 01) and used the results to guide analyses of additional families (Families 02–11). Four principal findings emerged. (1) A locus for GTCS was identified in Family 01 at 10q25–q26. Model-based multipoint analysis showed a LOD score of 2.85; sim-

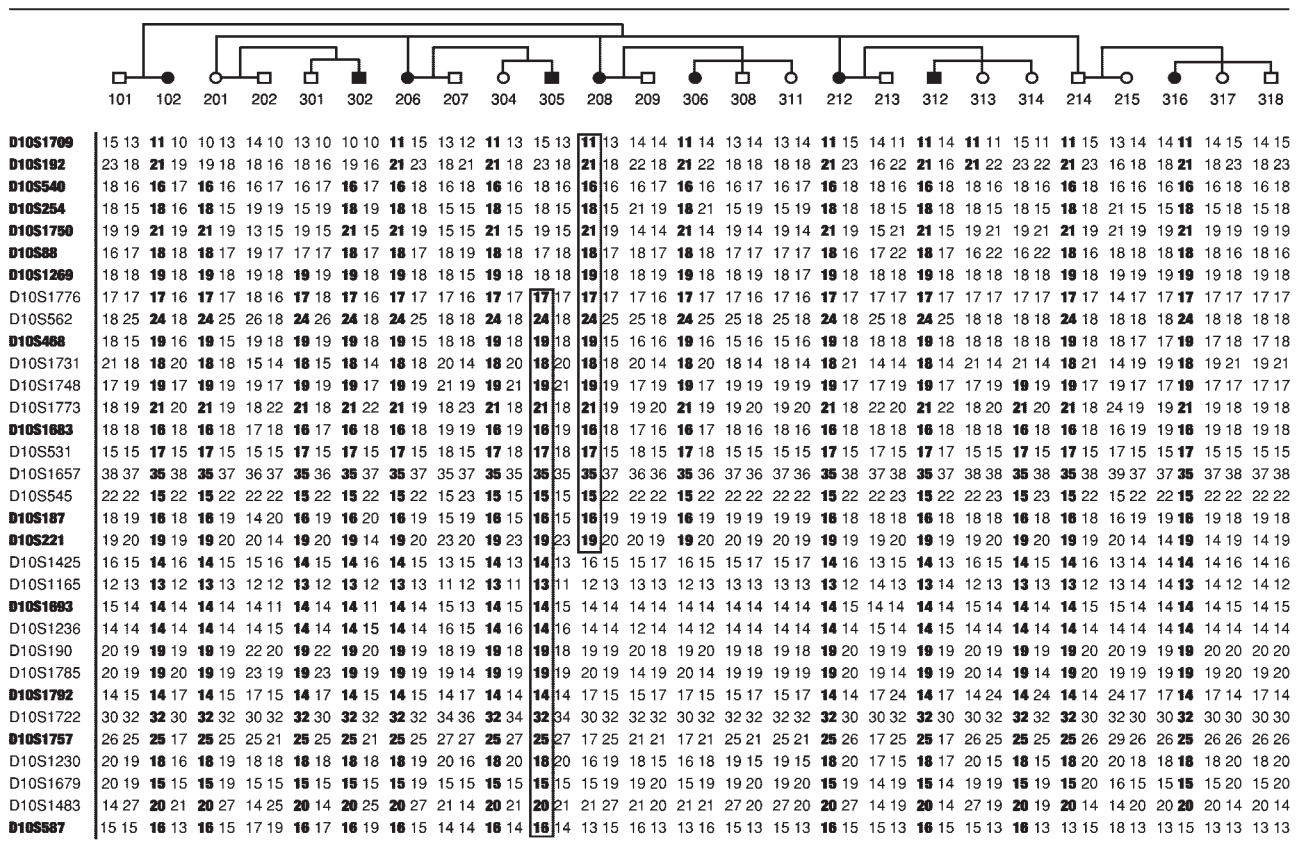


Fig 3. Fine mapping and haplotype analysis of the generalized tonic-clonic seizures locus on chromosome 10 in Family 01. All twenty-five members were genotyped with 32 markers shown in the figure. Haplotype analysis was conducted using SimWalk2.89, and the affected haplotype is shown in boldface. Recombination events in Subject 01-208 between the markers D10S221 and D10S1425 defined the telomeric boundary of the locus. The recombination event in Subject 01-305 between the markers D10S1269 and D10S1776 defined the centromeric boundary of the locus. This distance between marker D10S1269 and D10S1425 was determined to be 4.82Mb (4.87cM).

ilar results were obtained with model-free analyses (maximum NPL of 2.71 [ $p = 0.0019$ ]). (2) Analyses of the 10q25-q26 locus performed in 10 additional families assuming heterogeneity showed evidence for linkage in four families. Model-based and model-free analyses showed a HLOD of 2.01 ( $\alpha = 0.41$ ) and maximum NPL of 2.56 ( $p = 0.0027$ ), respectively, for affection status C. (3) Combined analyses of all 11 families showed a HLOD score of 4.04 ( $\alpha = 0.51$ ) and maximum NPL value of 4.20 ( $p = 0.0$

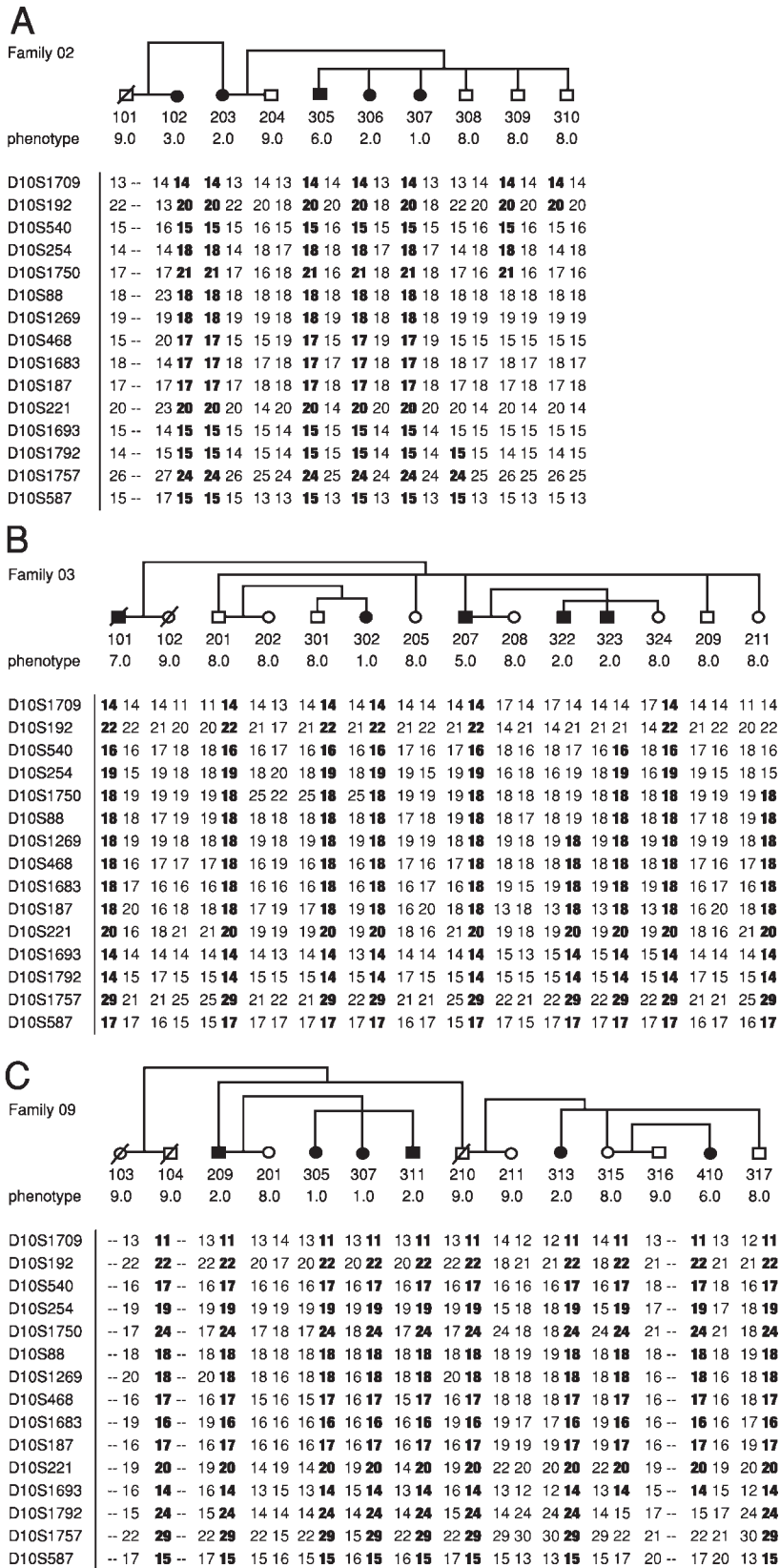


Fig 4. Haplotype analysis of the *GTCS* locus on chromosome 10 in linked Families 02 (A), 03 (B), and 09 (C). The subject identification number and the corresponding phenotype are shown below each subject, respectively. The 15-marker panel shown was used to genotype all available members from the above families. Data for the deceased subjects and Subjects 02-204 (A) and 09-211 and 09-316 (C) was inferred from identity-by-descent to alleles which are known. Individuals 02-305 (phenotype 6.0, A), 03-207 (phenotype 5.0, B), and 09-410 (phenotype 6.0, C) all share the same haplotype as the other affected subjects in their families.

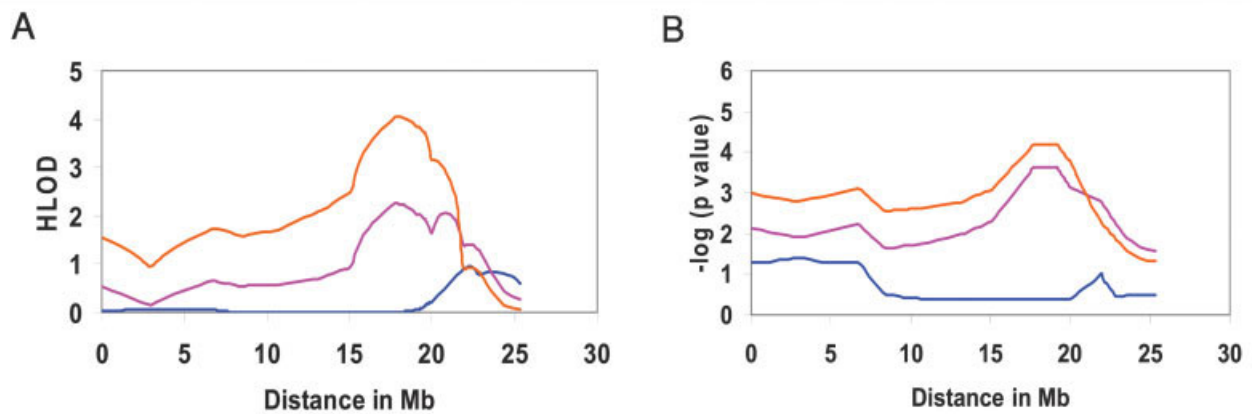


Fig 5. (A) Multipoint heterogeneity logarithm of odds (HLOD) scores under different affection statuses for all the families including 01. (B) Multipoint nonparametric  $-\log(p)$  value distribution under different affection statuses for all the families, including 01. The blue, magenta, and orange lines correspond to affection status A, B, C, respectively.

four subjects were not linked to 10q25-q26, then designating them as affected (affection status C) should reduce evidence for linkage; alternatively, if GTCS were linked to 10q25-q26, then designating them as affected should increase evidence for linkage. The increased HLOD (affection status B 0.37, affection status C 2.01) and NPL (affection status B 1.74, affection status C 2.56) scores with affection status C compared with B suggests that the 10q25-q26 locus confers susceptibility to GTCS whether generalized from the outset or secondarily generalized. Importantly, when all 11 families are considered together assuming heterogeneity, model-free analyses provide evidence for linkage whether affection status is designated B (NPL 3.62) or C (NPL 4.20) (see Fig 5B).

Previous authors suggested that a given locus may confer susceptibility to a single type of seizure and that inheritance of multiple loci may be required for expression of a syndrome with multiple types of seizures. This suggestion emerged from a genome-wide survey for linkage of 91 families with diverse IGE syndromes.<sup>15</sup> Among the multiple models examined, one designated affected as those families with JME and another designated affected as those families without JME; a locus on 6p21 was detected in the families with JME but not in the families with IGE without JME. Although these experiments designated affection status on the basis of a syndrome (JME) not a seizure type (ie, myoclonic seizures or GTCS), the authors speculated that a given locus may confer susceptibility to a specific type of seizure. This proposal found support from Winawer and colleagues,<sup>16</sup> who evaluated 34 families with 2 or more members with an IGE syndrome and determined that the number of families concordant for myoclonic seizures exceeded that expected by chance. Likewise, the number of families concordant for absence seizures exceeded that expected by chance. Although this experimental design does not

distinguish genetic from environmental factors, the data are consistent with the conclusion that distinct loci confer susceptibility to myoclonic and absence seizures, respectively.

Have any of the susceptibility genes or loci implicated in JME been shown to confer susceptibility to a single type of seizure? A nonsynonymous substitution in an  $\alpha$  subunit of a GABA<sub>A</sub> receptor has been identified by Cossette and colleagues,<sup>25</sup> in an autosomal dominant form of JME in a single large family, the molecular phenotype being a loss of function as defined in an *in vitro* recombinant system. In contrast with the families of this study, each of the eight affected members of this four-generation family exhibited JME and no other IGE syndrome. Haug and colleagues<sup>26</sup> identified mutations of CLCN2 in 3 of 46 families linked to 3q26, the forms of IGE including CAE, JAE, JME, and GTCS. Likewise, Suzuki and colleagues<sup>27</sup> discovered mutations of EFGH1 in 6 of 44 families in which the proband had JME and linkage to 6p11 had been established; some affected members of these families exhibited JME and others exhibited non-JME forms of IGE. After establishing linkage of families with JME to 6p21,<sup>15</sup> association studies by Pal and colleagues<sup>5</sup> identified two JME-associated SNP variants in the promoter region of BRD2. Likewise, after establishing linkage of a locus at 18q21 with multiple IGE syndromes, association studies led to identification of a nine SNP haplotype in malic enzyme 2 (ME2) that was associated with increased risk of IGE.<sup>6</sup> Apart from the genes identified above, a locus at 15q14 has been identified in families in which the proband exhibits JME.<sup>28</sup> In none of the above studies was a gene or locus demonstrated to confer susceptibility to a single type of seizure. The findings by Cossette and colleagues<sup>25</sup> demonstrate that a mutation of a single gene can be sufficient in some instances to confer susceptibility to the multiple types of seizures evident in

an IGE syndrome such as JME. With respect to the other studies, none has been designed in such a way to permit testing the relationship to a specific type of seizure. For example, the method of designating affected used by Pal and colleagues,<sup>5</sup> permits examining association with the syndrome of JME but does not assess the relationship between BRD2 and susceptibility specifically to myoclonic and/or GTCS. Likewise, the findings of Haug and colleagues<sup>26</sup> suggest that mutation of a single gene can be sufficient to confer susceptibility to multiple IGE syndromes, each of which can have multiple types of seizures.

In contrast with each of the above studies, this work designated subjects as affected by virtue of a type of seizure, not by a syndrome consisting of multiple types of seizures. This led to the first demonstration of a seizure-specific locus, the locus segregating with GTCS, in particular. Had we simply designated all members of these families with some form of IGE as affected (eg, affection status B), evidence for linkage would have been detected. However, this would have obscured the relationship to GTCS, in particular, and the potential relationship to secondarily generalized GTCS. In addition, the possible locus segregating with myoclonic seizures on chromosome 16 in Family 01 would have been missed. Although the suggested chromosome 16 locus together with the 10q25-q26 loci are consistent with the seizure-specific two-locus hypothesis underlying the syndrome of JME, our findings are consistent with an alternative hypothesis. Although myoclonic seizures in the absence of associated GTCS are occasionally seen in relatives of JME probands (eg, Families 06 and 10 of this study), this phenotype (1.5) was not observed in Family 01. This raises the possibility that the chromosome 16 locus, if confirmed, contains a modifier gene; thus inheritance of the modifier gene alone might result in no phenotype (as seen in Subject 01-101) but when inherited together with the GTCS locus transforms the phenotype from GTCS alone to both myoclonic and GTCS (JME).

We have reviewed the various genes contained within the 4.45Mb interval and considered their candidacy for conferring susceptibility to GTCS. The critical interval identified in this work harbors 33 genes, and more than 95 putative genes in which an open reading frame has been identified by Genescan analysis. With respect to susceptibility genes for GTCS, proteins controlling propagation of partial into GTCS may provide a clue. Pharmacological and genetic analyses of seizure models demonstrate that the activation of  $\alpha_2$  adrenergic receptors on targets of noradrenergic neurons of the locus ceruleus powerfully controls the transition of a partial into a secondarily generalized tonic-clonic seizure.<sup>29,30</sup> Thus, the vesicular monoamine transporter SLC18A2 warrants consideration. The evidence implicating mutations of diverse ion channels in

episodic disorders of multiple organs, including epilepsy in particular, underscores the potential importance of a recently identified potassium channel, TWIK-related spinal cord K<sup>+</sup> channel that is highly expressed in brain.<sup>31</sup> The identification of epilepsy genes that are not known to be directly related to synapses or control of intrinsic neuronal excitability<sup>22,32,33</sup> expands the list of possible candidate genes.

## Disclosure

This work was conducted as a part of the "AIMS-DUKE University Collaborative Project Linkage studies on families with probands exhibiting Juvenile Myoclonic Epilepsy, Idiopathic Generalized Epilepsy and other Epileptic Syndromes-Project Code: NI-362/98." The data designated "not shown" will be provided upon request.

---

This work was supported by grants from the Department of Veterans Affairs (J.O.M.), an unrestricted research grant from the Bristol-Myers Squibb Foundation (J.O.M.), the U.S. Public Health Service Resource (RR03655, R.C.E.), the National Center for Research Resources (GM28356, R.C.E.), and the National Institute of General Medical Sciences (R.C.E.).

We thank the family members for their participation in this study, Drs S. F. Berkovic and D. Goldstein for comments on the manuscript, and Dr M. Dupree for help in troubleshooting ABI-Prism 310-based genotyping.

---

## References

1. Scheffer IE, Berkovic SF. The genetics of human epilepsy. *Trends Pharmacol Sci* 2003;24:428–433.
2. Steinlein OK. Genetic mechanisms that underlie epilepsy. *Nat Rev Neurosci* 2004;5:401–408.
3. Robinson R, Gardiner M. Molecular basis of Mendelian idiopathic epilepsies. *Ann Med* 2004;36:89–97.
4. Gourfinkel-An I, Baulac S, Nabbout R, et al. Monogenic idiopathic epilepsies. *Lancet Neurol* 2004;3:209–218.
5. Pal DK, Evgrafov OV, Tabares P, et al. BRD2 (RING3) is a probable major susceptibility gene for common juvenile myoclonic epilepsy. *Am J Hum Genet* 2003;73:261–270.
6. Greenberg DA, Cayanis E, Strug L, et al. Malic enzyme 2 may underlie susceptibility to adolescent-onset idiopathic generalized epilepsy. *Am J Hum Genet* 2005;76:139–146.
7. Greenberg DA, Delgado-Escueta AV. The chromosome 6p epilepsy locus: exploring mode of inheritance and heterogeneity through linkage analysis. *Epilepsia* 1993;34(S3):S12–S18.
8. Berkovic SF, Howell RA, Hopper JL. Twin study of epilepsy syndromes. *Epilepsia* 1993;34(S2):38.
9. Berkovic SF, Howell RA, Hay DA, Hopper JL. Epilepsies in twins: genetics of the major epilepsy syndromes. *Ann Neurol* 1998;43:435–445.
10. Marini C, King MA, Archer JS, et al. Idiopathic generalized epilepsy of adult onset: clinical syndromes and genetics. *J Neurol Neurosurg Psychiatry* 2002;74:192–196.
11. Bianchi A, Viaggi S, Chiossi E, the LICE Episcreeen Group. Family study of epilepsy in first degree relatives: data from Italian Episcreeen Study. *Seizure* 2004;12:203–210.
12. Hauser WA, Hesdorffer DC. *Epilepsy: frequency, causes and consequences*. New York: Demos Press, 1990.

13. Italian League Against Epilepsy Genetic Collaborative Group. Concordance of clinical forms of epilepsy in families with several affected members. *Epilepsia* 1993;34:819–826.
14. Jain S, Dixit SN, Andrew PI, et al. Disease expression among probands with juvenile myoclonic epilepsy and their family members in two population groups. *J Epilepsy* 1996;9:259–267.
15. Durner M, Keddache MA, Tomasini L, et al. Genome scan of idiopathic generalized epilepsy: evidence for major susceptibility gene and modifying genes influencing the seizure type. *Ann Neurol* 2001;49:328–335.
16. Winawer MR, Rabinowitz D, Pedley TA, et al. Genetic influences on myoclonic and absence seizures. *Neurology* 2003;61:1576–1581.
17. Chen X, Levine L, Kwok P. Fluorescence polarization in homogenous nucleic acid analysis. *Genome Res* 1999;9:492–498.
18. Sobel E, Lange K. Descent graphs in pedigree analysis: applications to haplotyping, location scores, and marker-sharing statistics. *Am J Hum Genet* 1996;58:1323–1337.
19. Lange K, Boehnke M. Some combinatorial problems of DNA restriction fragment length polymorphisms. *J Hum Genet* 1983;35:177–192.
20. Lange K, Goradia TM. An algorithm for automatic genotype elimination. *Am J Hum Genet* 1987;40:250–256.
21. Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES. Parametric and nonparametric linkage analysis: a unified multipoint approach. *Am J Hum Genet* 1996;58:1347–1363.
22. Kalachikov S, Evgrafov O, Ross B, et al. Mutations in LGI1 cause autosomal dominant partial epilepsy with auditory features. *Nat Genet* 2002;30:335–341.
23. Greenberg DA, Durner M, Keddache M, et al. Reproducibility and complications in gene searches: linkage on chromosome 6, heterogeneity, association, and maternal inheritance in juvenile myoclonic epilepsy. *Am J Hum Genet* 2000;66:508–516.
24. Meisler MH, Kearney J, Ottman R, Escayg A. Identification of epilepsy genes in human and mouse. *Annu Rev Genet* 2001;35:567–588.
25. Cossette P, Liu L, Brisebois K, et al. Mutation of GABRA1 in an autosomal dominant form of juvenile myoclonic epilepsy. *Nat Genet* 2002;31:184–189.
26. Haug K, Warnstedt M, Alekov AK, et al. Mutations in CLCN2 encoding a voltage gated chloride channel are associated with idiopathic generalized epilepsies. *Nat Genet* 2003;33:527–532.
27. Suzuki T, Delgado-Escueta AV, Aguan K, et al. Mutations in EFHC1 cause juvenile myoclonic epilepsy. *Nat Genet* 2004;36:842–849.
28. Elmslie FV, Rees M, Williamson MP, et al. Genetic mapping of a major susceptibility locus for juvenile myoclonic epilepsy on chromosome 15q. *Hum Mol Genet* 1997;6:1329–1334.
29. Gellman RL, Kallianos JA, McNamara JO. Alpha<sub>2</sub> receptors mediate an endogenous noradrenergic suppression of kindling development. *J Pharmacol Exp Ther* 1987;241:891–898.
30. Janumpalli S, Butler LS, MacMillan LB, et al. A point mutation (D79N) of the  $\alpha_{2A}$  adrenergic receptor abolishes the anti-epileptogenic action of endogenous norepinephrine. *J Neurosci* 1998;18:2004–2008.
31. Sano Y, Inamura K, Miyake A, et al. A novel two-pore domain K<sup>+</sup> channel, TRESK, is localized in the spinal cord. *J Biol Chem* 2003;278:27406–27412.
32. Skradski SL, Clark AM, Jiang H, et al. A novel gene causing a mendelian audiogenic mouse epilepsy. *Neuron* 2001;31:537–544.
33. Nakayama J, Fu YH, Clark AM, et al. A nonsense mutation of the MASS1 gene in family with febrile and afebrile seizures. *Ann Neurol* 2002;52:654–657.