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Mammalian Genome
Genes and Phenotypes

Genetic modifiers affecting severity of epilepsy caused by mutation of sodium channel *Scn2a*

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Abstract

Mutations in the voltage-gated sodium channels *SCN1A* and *SCN2A* are responsible for several types of human epilepsy. Variable expressivity among family members is a common feature of these inherited epilepsies, suggesting that genetic modifiers may influence the clinical manifestation of epilepsy. The transgenic mouse model $Scn2a^{Q54}$ has an epilepsy phenotype as a result of a mutation in *Scn2a* that slows channel inactivation. The mice display progressive epilepsy that begins with short-duration partial seizures that appear to originate in the hippocampus. The partial seizures become more frequent and of longer duration with age and often induce secondary generalized seizures. Clinical severity of the $Scn2a^{Q54}$ phenotype is influenced by genetic background. Congenic C57BL/6J.Q54 mice exhibit decreased incidence of spontaneous seizures, delayed seizure onset, and longer survival in comparison with [C57BL/6J × SJL/J]F_1.Q54 mice. This observation indicates that strain SJL/J carries dominant modifier alleles at one or more loci that determine the severity of the epilepsy phenotype. Genome-wide interval mapping in an N_2 backcross revealed two modifier loci on Chromosomes 11 and 19 that influence the clinical severity of of this sodium channel-induced epilepsy. Modifier genes affecting clinical severity in the $Scn2a^{Q54}$ mouse model may contribute to the variable expressivity seen in epilepsy patients with sodium channel mutations.

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Introduction

Mutations in voltage-gated sodium channels associated with human epilepsy were first identified in the disorder Generalized Epilepsy with Febrile Seizures Plus (GEFS+) (Wallace et al. 1998; Escayg et al. 2000). GEFS+ is a heterogeneous syndrome in which affected family members have febrile seizures in childhood which may progress to other seizure types later in life. More than 150 mutations in the sodium channels *SCN1A* and *SCN2A* have been identified in human patients (reviewed in Meisler and Kearney 2005). The variable expressivity among family members with the same primary mutation suggests that the clinical severity depends on secondary factors which may include genetic modifiers.

We have developed a mouse model with a missense mutation of *Scn2a*, designated $Scn2a^{Q54}$, with an epilepsy phenotype that resembles human patients (Kearney et al. 2001). $Scn2a^{Q54}$ mice express a transgene in which expression of the $Scn2a^{GAL879-881QQQ}$ cDNA (abbreviated $Scn2a^{Q54}$) is driven by the neuron-specific enolase (NSE) promoter. The mutation GAL879-881QQQ in the cytoplasmic S4-S5 linker of domain 2 results in delayed channel inactivation and increased persistent current when measured in *Xenopus* oocytes and in hippocampal neurons from $Scn2a^{Q54}$ transgenic mice (Smith and Goldin 1997; Kearney et al. 2001). The level of persistent current in $Scn2a^{Q54}$ neurons (~2% of total peak current) is comparable to that produced by mutations such as $SCN1A^{R1648H}$ in families with GEFS+ (Lossin et al. 2002).

$Scn2a^{Q54}$ transgenic mice exhibit progressive epilepsy that begins at 6-8 weeks of age with recurrent, spontaneous partial seizures that appear to originate in the hippocampus (Kearney et al. 2001). The electrographic seizures are accompanied by episodes of behavioral arrest, tonic deviation of the head

and body, and forelimb clonus (Kearney et al. 2001). *Scn2a*^{Q54} transgenic mice exhibit hippocampal pathology that resembles human mesial temporal lobe epilepsy with neuron loss in areas CA1 and CA3 and in the hilus of the dentate gyrus (Kearney et al. 2001). The GEFS+ spectrum includes temporal lobe epilepsy (Abou-Khalil et al. 2001). Line C57BL/6J.Q54 was established by backcrossing a (C57BL/6J × SJL/J)F_2 founder to strain C57BL/6J. Offspring from the first and second backcross generations (N_1 and N_2) were used to characterize the phenotype described in Kearney et al. (2001). Twelve percent to 25% of the genome of these mice is derived from strain SJL/J.

We have established the congenic line C57BL/6J.Q54 (abbreviated B6.Q54) by ten generations of backcrossing to strain B6. This line is estimated to contain less than 0.1% of SJL-derived DNA (Silver 1995). We describe a strain difference in the *Scn2a*^{Q54} phenotype between the congenic B6.Q54 line and the (B6 × SJL/J)F_1 background. This suggests that strain SJL/J carries alleles at one or more modifier loci that increase the incidence and severity of seizures caused by the *Scn2a*^{Q54} mutation. We report the results of analysis of an N_2 backcross that reveals two modifier loci that influence epilepsy susceptibility due to a sodium channel mutation.

Materials and methods

Mice. *Scn2a*^{Q54} transgenic mice (TgN54Mm) were generated by microinjection of (C57BL/6J × SJL/J)F_2 oocytes as described (Kearney et al. 2001). The congenic line C57BL/6J.Q54 (abbreviated B6.Q54) was established by ten successive generations of backcrossing to strain C57BL/6J and is maintained by continued backcrossing of hemizygous transgenic males to C57BL/6J females. Strain SJL/J was crossed to B6.Q54 to generate (B6.Q54 × SJL)F_1 offspring. For mapping, (B6.Q54 × SJL)F_1 mice were backcrossed to strain C57BL/6J. SJL/J (stock No. 00686) and C57BL/6J (stock No. 00064) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). The *doublerridge* (*dblr*) mouse mutant was identified in a screen for insertional mutants and found to be caused by a transgene insertion on the SJL/J-derived Chromosome 19 of the founder (MacDonald et al. 2004). The incipient congenic line B6.*dblr* with the interval of Chromosome 19 containing the SJL/J-derived *dblr* mutation has been backcrossed to C57BL/6J for eight generations and is estimated to contain less than 2% of SJL-derived DNA (Silver 1995).

Phenotyping. Mice were genotyped for the Q54 transgene on postnatal day 14. Nontransgenic mice were discarded. *Scn2a*^{Q54} transgenic mice were observed for visible seizures during 30-min observation periods at various ages. N_2 backcross mice were observed for 30-min sessions at 3 and 6 weeks of age. Observations were performed between 1:00 and 4:00 pm in a temperature- and humidity-controlled room. Mice were transferred to a clean observation cage ($7\frac{3}{4}$ in. W × 12 in. D × $6\frac{1}{2}$ in. H) just before the observation session. Assessment of visible seizures was based on prior video-EEG monitoring that demonstrated coincident behavioral and EEG abnormalities (Kearney et al. 2001).

Genotyping. DNA was prepared from tail biopsies by phenol:chloroform extraction and ethanol precipitation. Q54 transgene-positive mice were identified by PCR with primers in exon 4 and exon 5 of *Scn2a* (Q54F-5′-GCAAGGGGCTTTTGTCTAG AAG-3′ and Q54R-5′-TCAATGCTCGGAGAACTC TGAACG-3′). These primers amplify a 715-bp genomic product from the endogenous *Scn2a* gene and a 151-bp product from the *Scn2a*^{Q54} cDNA transgene. Genome-wide microsatellite genotyping services were provided by the Center for Inherited Disease Research (CIDR, http://www.cidr.jhmi.edu/mouse/mouse_strp.html). (CIDR is fully funded through a federal contract from the National Institutes of Health to The Johns Hopkins University, Contract Number N01-HG-65403.) Additional microsatellite genotyping was performed by analysis of PCR products on 7% nondenaturing polyacrylamide gels stained with ethidium bromide or an ABI 3730 Automated Sequencer (Applied Biosystems, Foster City, CA) in The University of Michigan DNA Sequencing Core (Robert Lyons, Director, http://seqcore.brcf.med.umich.edu). SNP genotyping was performed by analysis of PCR products by conformation-sensitive gel electrophoresis (CSGE) as described (Plummer et al. 1998) or by sequencing. The *dblr* and *Dkk1*$^{+/-}$ mice were genotyped as described (MacDonald et al. 2004).

Genetic analysis. Association between genotypes and the presence of epilepsy at each locus was first assessed by single-marker analysis using logistic regression, wherein the significance of shifts in allelic proportions was determined by an odds-ratio test. We then used interval mapping, using a maximum-likelihood procedure designed for categorical traits (Galecki et al. 2001). We refer to this method as categorical trait interval mapping (CTIM). CTIM takes as its underlying model an example of a latent class model in which a categorical (here, dichotomous) phenotype is treated as a function of both the unknown (unobservable) genotype at a putative marker and the known (observable) geno-

type at flanking markers. Under this model each probability defining the joint distribution of phenotype data *and* unobservable genotypes (complete data) is decomposed into the product of two underlying probabilities: Prob(phenotype|unobservable genotype) × Prob(unobservable genotype). Component probabilities are then modeled using logistic regression and Haldane's mapping function, respectively. This model may also be considered an elaboration of generalized linear models, with a composite link function applied to odds ratios and binomial distribution (McCullagh and Nelder 1989). This methodology implemented in Preisser et al. (2000) and Ayyadevara et al. (2003) uses maximum-likelihood calculations implemented with SAS module PROC NLIN, with a separate SAS macro that allows permutation test calculations. A working version of PROC NLIN code is available at http://www-personal.umich.edu/~agalecki/ via a link to ''CTIM: a set of SAS macros for categorical trait interval mapping.''

Significance of single-marker peaks can be evaluated in terms of the expected incidence of false positives (type I errors). In single-marker analysis, the LOD score (and corresponding χ^2) threshold for an experiment-wise α value of 0.05 (i.e., a 5% chance of obtaining at least one association of marker to trait, purely by chance, anywhere in the genome) was estimated empirically (Churchill and Doerge 1994) by determining the χ^2 statistics for each marker over 1000 permutations of the trait category assigned to each genotype.

Candidate gene screening. Positional candidate genes were identified from the publicly available mouse genome sequence (www.ensembl.org) and selected by tissue expression and gene function. Primers were designed using Oligo Primer Analysis software (ver. 6.65, Molecular Biology Insights, Cascade, CO). Genes were amplified from B6 and SJL by RT-PCR or by PCR of exon sequence from genomic DNA. PCR products were gel-purified and sequenced on an ABI 3730 Automated Sequencer in The University of Michigan DNA Sequencing Core Facility. Sequences were compared using Sequencher software (GeneCodes, Ann Arbor, MI).

Results

Effect of genetic background. *Scn2a*^{Q54} transgenic mice were generated by microinjection of (C57BL/6J × SJL/J)F_2 eggs as previously described (Kearney et al. 2001). The initial characterization used mice from the first (N_1) and second (N_2) C57BL/6J backcross generations with 12%–25% of the genome derived from strain SJL/J (Kearney et al. 2001). We have carried out ten successive generations of backcrossing to transfer the *Scn2a*^{Q54} transgene onto the C57BL/6J background, establishing the congenic line C57BL/6J.Q54 (abbreviated B6.Q54). This line is estimated to contain less than 0.1% of SJL/J-derived DNA (Silver 1995). The phenotype of the congenic line was found to be much less severe than in the N_1 and N_2 mice, with low incidence, delayed onset and reduced severity of seizures, and increased longevity.

To determine whether the reduced severity resulted from loss of SJL/J alleles at modifier loci, we crossed the B6.Q54 congenic mice to strain SJL/J. The resulting (SJL × B6)F_1.Q54 offspring (abbreviated F_1.Q54) were genotyped at P14 and observed in 30-min sessions at 3, 4.5, and 6 weeks of age. A comparison of the phenotype in F_1.Q54 and B6.Q54 mice is presented in Fig. 1. F_1.Q54 mice exhibited high incidence of seizures (>80%) with early onset (Fig. 1A). In contrast, B6.Q54 mice exhibited a low incidence of seizures, with less than 20% having seizures at 3 months of age (Fig. 1A). Affected F_1 animals have a high seizure frequency of 8-16 per 30 min, while seizure frequency is low in the small number of B6.Q54 mice that exhibit seizures (Fig. 1B). Fewer than 25% of F_1.Q54 mice survive to 6 months of age compared with greater than 75% of the congenic B6.Q54 mice (Fig. 1C). The data indicate that strain SJL/J carries alleles at one or more loci that influence susceptibility to epilepsy caused by the *Scn2a* mutation.

Backcross mapping of modifier loci. To localize loci responsible for the strain difference in seizure susceptibility between B6.Q54 and F_1.Q54 mice, we carried out a backcross of F_1.Q54 mice to strain C57BL/6J. Backcross progeny carrying the *Scn2a*^{Q54} transgene were examined by visual observation for 30 min at 3 and 6 weeks of age. Mice that exhibited one or more seizures during the 30-min observation were classified as ''susceptible'' and mice without seizures as ''resistant.'' Among 150 backcross animals there were 46 susceptible and 104 resistant individuals. The deviation from a 1:1 ratio ($\chi^2 = 22.4$, $p < 2.1e^{-6}$) indicates that there is more than one locus responsible for the phenotype difference between strains. This seizure incidence in the N_2 backcross population is intermediate between the parental strains (Fig. 3).

A 20-cM-resolution genome scan, including 90 microsatellite markers with an average spacing of 14.9 cM, was carried out on 79 backcross DNAs, including 38 susceptible and 41 resistant. Genotyping of additional N_2 animals was performed on chromosomes with significant association by single-

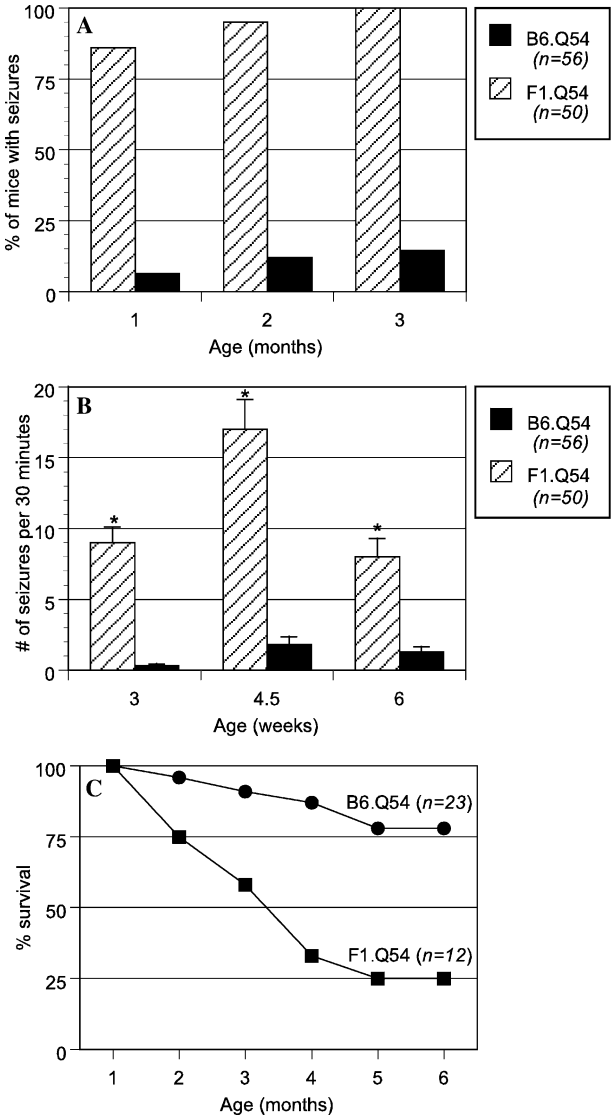

Fig. 1. Severity of epilepsy in F_1.Q54 and B6.Q54 mice. **A**. Incidence of seizures in F_1.Q54 and B6.Q54 mice at 1–3 months of age. **B**. Average number of seizures in F_1.Q54 and B6.Q54 mice at 3, 4.5, and 6 weeks of age ($^{\star}p < 1e^{-9}$). **C**. Reduced lifespan in F_1.Q54 mice compared with that of B6.Q54.

marker analysis. The *Scn2a*^{Q54} transgene insertion site was localized to mouse Chromosome 1 between 58 and 70 cM (94–103 Mb), reducing the ability to detect modifiers on this chromosome.

Modifier loci affecting epilepsy susceptibility resulting from a sodium channel mutation were mapped using CTIM analysis. The genome-wide significance threshold of $p = 0.05$ corresponding to a LOD score of 2.6 was determined with 1000 permutations of the trait data with respect to observed genotypes. CTIM analysis provided suggestive or significant evidence for two modifier loci on Chromosomes 11 and 19 (Fig. 2). The locus on

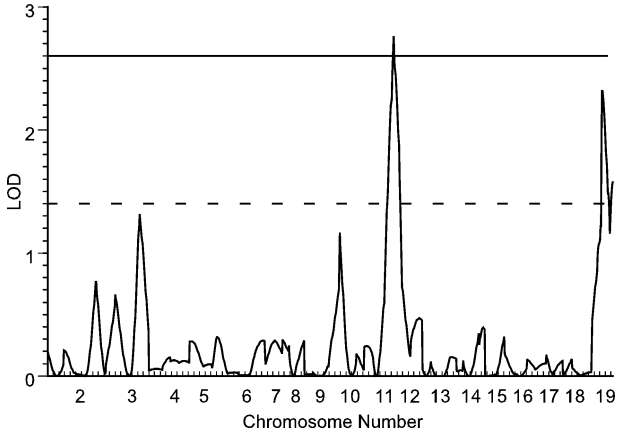

Fig. 2. Genome-wide interval mapping for epilepsy susceptibility due to a sodium channel mutation. The *Y* axis indicates the LOD score. Horizontal lines mark the significance thresholds for genome-wide suggestive (LOD = 1.4, $p < 0.63$; dashed line) and significant (LOD = 2.6, $p < 0.05$; solid line) evidence of linkage based on 1000 permutations of the data. The *X* axis is in centimorgans with chromosome numbers indicated below.

Chromosome 11, designated *Moe1* (modifier of epilepsy 1), peaks at 54 cM (95 Mb). Inheritance of an SJL allele at this locus confers increased resistance. Among the resistant N_2 mice, 67% (53/80; $\chi^2 = 8.45$, $p < 0.004$) inherited an SJL allele at *D11Mit289*. The locus on Chromosome 19 designated *Moe2* peaks at 24 cM (31.3 Mb) and inheritance of an SJL allele at this locus confers increased susceptibility. Among the susceptible N_2 mice, 67% (31/46; $\chi^2 = 5.57$, $p < 0.02$) inherited an SJL allele at *D19Mit135*. No significant epistatic interactions were detected between *Moe1* or *Moe2* and other loci in the genome.

The combined effect of *Moe1* and *Moe2* accounts for approximately 80% of the difference in seizure incidence between B6.Q54 and F_1.Q54 mice. Inheritance of three susceptible alleles, including two B6 alleles at *Moe1* and an SJL allele at *Moe2*, results in seizure incidence approaching that of the F_1.Q54 mice (Fig. 3). Conversely, inheritance of one susceptible allele and three resistant alleles at these loci (*Moe1*^{BS}, *Moe2*^{BB}) results in seizure incidence similar to B6.Q54 mice (Fig. 3).

Chromosome 19 interval-specific strain. The B6.*dblr* mouse strain carries an SJL/J-derived segment of Chromosome 19 that spans the *Moe2* 1-LOD support interval from *D19Mit86* to *D19Mit88* (Fig. 4A). The *dblr* mutation was caused by a transgene insertion with a 60-kb intergenic deletion of mouse Chromosome 19, resulting in decreased expression of the Wnt signaling inhibitor *Dkk1* (MacDonald et al. 2004). Mice homozygous for the

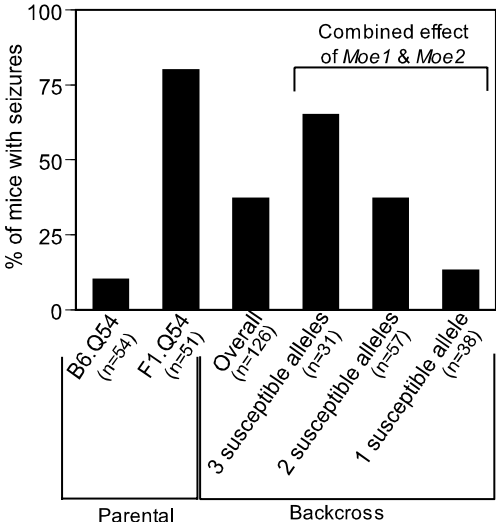

Fig. 3. Combined effect of *Moe1* and *Moe2* on the incidence of seizures in backcross offspring. Backcross animals were grouped according to the number of susceptible alleles inherited at the peak markers of the *Moe1* (*D11Mit289*) and *Moe2* (*D19Mit135*) loci. Susceptible allele: $Moe1^B$, $Moe2^S$. The incidence of seizures for each group is shown. The overall bar represents seizure incidence in the backcross population without grouping by genotype. Incidence of seizures in the parental strains is provided for comparison.

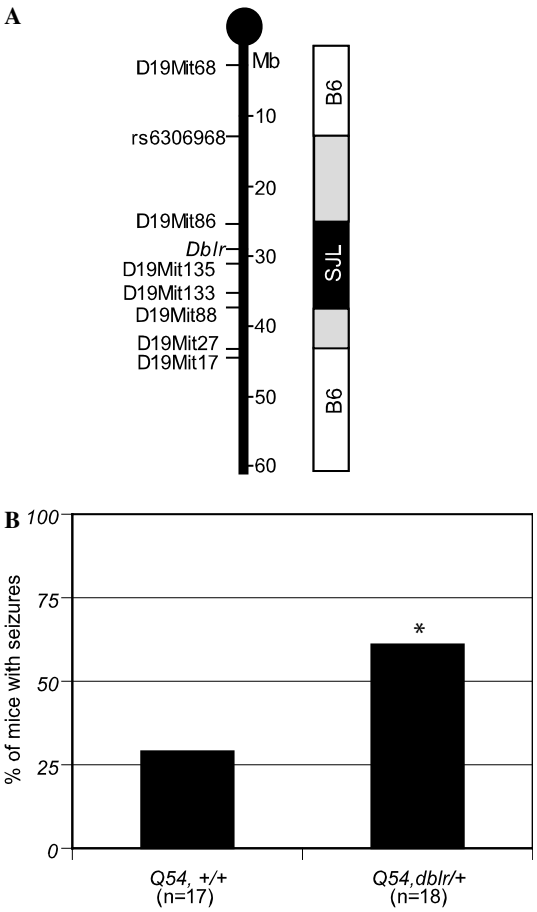

Fig. 4. Effect of an SJL-derived interval of Chromosome 19 on epilepsy phenotype in B6.Q54 congenic mice. **A**. Map of Chromosome 19 showing the SJL/J-derived interval in *dblr* mice. The SJL/J-derived interval is shown in black. Gray denotes the interval containing the recombination. **B**. Incidence of seizures in offspring of B6.Q54 mice crossed to B6.*dblr*/+ mice. Mice with the SJL-derived interval of Chromosome 19 are indicated as (Q54, *dblr*/+) and littermates that are B6 in the interval are indicated as (Q54, +/+) (*$p < 0.004$).

dblr mutation have defects in forelimb development (Adamska et al. 2003). Heterozygous *dblr*/+ mice do not display spontaneous seizures or increased susceptibility to seizures induced by pentylenetetrazol (40 and 50 mg/kg i.p.) (data not shown).

To confirm the mapping of the *Moe2* locus on Chromosome 19, we crossed B6.*dblr*/+ mice with B6.Q54 mice. Offspring were genotyped for $Scn2a^{Q54}$, the *dblr* mutation, and the microsatellite markers *D19Mit86*, *D19Mit135*, and *D19Mit133*. $Scn2a^{Q54}$ transgene-positive offspring underwent 30-min observations for spontaneous seizures at 3, 4.5, and 6 weeks of age. B6.Q54 mice carrying the SJL-derived *dblr* interval of Chromosome 19 exhibited a higher incidence of spontaneous seizures than B6.Q54 mice ($\chi^2 = 8.3$, $p < 0.004$) (Fig. 4B). The increased incidence of seizures in (B6.Q54, *dblr*/+) mice supports the mapping of *Moe2* to Chromosome 19.

To test the effect of reduced levels of *Dkk1* on seizure susceptibility, we crossed B6.Q54 mice with $Dkk1^{+/-}$ null heterozygotes (Mukhopadhyay et al. 2001). (B6.Q54, $Dkk1^{+/-}$) mice do not exhibit increased seizure incidence compared with the (B6.Q54, $Dkkl^{+/+}$) littermates (data not shown), indicating that reduced expression of Dkk1 does not account for the increased seizure susceptibility of (B6.Q54, *dblr*/+) mice.

Positional candidate genes. The *Moe1* and *Moe2* regions were examined for strong candidate genes in the publicly available mouse genome sequence (www.ensembl.org). In the *Moe1* region of Chromosome 11, candidate genes included two inwardly rectifying potassium channels (*Kcnj2, Kcnj16*) and seizure-related gene 6 (*Sez6*). In the *Moe2* region on Chromosome 19, candidate genes included a high-affinity glutamate transporter (*Slc1a1*), a serotonin receptor (*Htr7*), the very-low-density lipoprotein receptor (*Vldlr*), and two tumor suppressor genes that have been associated with seizures in mice (*Pten*) (Backman et al. 2001) and human patients (*Lgi1*) (Kalachikov et al. 2002). We screened for coding sequence polymorphisms in these candidate genes by RT-PCR or by PCR of ex-

onic sequence from B6 and SJL genomic DNA. No coding sequence polymorphisms were observed. Regulation of expression was not evaluated.

Discussion

We report an effect of strain background on the severity of epilepsy caused by a sodium channel mutation. Genetic analysis of an N_2 backcross identified two modifier loci that influence the severity of epilepsy in *Scn2a*^{Q54} mice. This is in agreement with previous studies that have shown seizure susceptibility and severity to be under genetic influence (Frankel et al. 1995; Ferraro et al. 1997, 1999; Legare et al. 2000; Legare and Frankel 2000; Ferraro et al. 2001, 2002, 2004).

The phenotyping of mice in our mapping backcross divided animals into two categories based on the presence or absence of seizures. The categorical nature of our data precluded the use of conventional interval mapping procedures which assume that trait data are continuous and normally distributed. Methods for analysis of such data include nonparametric interval mapping, which makes no assumptions about the distribution of the trait data (Kruglyak and Lander 1995), and categorical traits interval mapping (CTIM), which is a Bayesian maximum-likelihood procedure for analysis of categorical traits (Galecki et al. 2001). Ayyadevara et al. (2003) performed a direct comparison of nonparametric interval mapping and CTIM in a study of lifespan of *C. elegans*. They found that both methods yielded consistent results but observed sharper peaks with CTIM mapping resulting in smaller 95% confidence intervals and assert that CTIM represents a more appropriate statistical model for categorical trait data (Ayyadevara et al. 2003).

Loci responsible for strain differences in susceptibility to seizures induced by handling, kainate, pentylenetetrazol, and electroconvulsive shock have been mapped to specific mouse chromosomes (Frankel et al. 1995; Ferraro et al. 1997, 1999; Legare et al. 2000; Legare and Frankel 2000; Ferraro et al. 2001, 2002, 2004). QTLs influencing seizure-induced cell death have also been mapped in strains C57BL/6J (resistant) and FVB/N (susceptible) (Schauwecker et al. 2004). Previous studies have shown that strain C57BL/6J is resistant to seizures induced by a variety of methods, including chemoconvulsants, electroconvulsive shock, audiogenic, and handling (Seyfried et al. 1980; Ferraro et al. 1997, 1999, 2001; Frankel et al. 2001; Ferraro et al. 2002; Kitami et al. 2004). Little has been reported about the seizure susceptibility of strain SJL/J. However, the limited reports on SJL and the demonstrated susceptibility of the closely related strain FVB/NJ suggest that SJL is likely to be on the susceptible end of the spectrum (Frankel et al. 2001; Golden et al. 2001; Chen et al. 2005).

A major-effect seizure susceptibility QTL (designated *Szs1*) has been mapped to distal Chromosome 1 by studies of strain differences in susceptibility to seizures induced by kainate, pentylenetetrazol, and maximal electroshock (Ferraro et al. 1997, 1999, 2001, 2002, 2004). Ferraro et al (2004) suggested that the potassium channel *Kcnj10* variant T262S is a likely candidate for *Szs1*. In support of this, a significant association was demonstrated between seizure resistance and the variant R271C in the human ortholog *KCNJ10* (Buono et al. 2004). In our N_2 backcross, *Szs1* segregated independent of seizure phenotype, indicating that it does not contribute to the observed difference in epilepsy severity between strains B6 and SJL.

Two previous studies identified seizure susceptibility QTLs on Chromosome 11 in regions overlapping the *Moe1* locus. QTL mapping in the epilepsy-susceptible EL mouse strain identified the locus *El6* on Chromosome 11 at which homozygosity for the allele from the resistant DDY/Jcl strain confers increased susceptibility to seizures (peak 68 cM) (Frankel et al. 1995). The QTL *Szs3* was mapped to the same region in a study of kainate-induced seizure susceptibility in strains DBA/2J and C57BL/6J (peak 66 cM). Homozygosity of the *Szs3* allele from the resistant B6 strain at this locus confers increased susceptibility to seizures (Ferraro et al. 1997). In our study, *Moe1* maps to the same region, and homozygosity for the allele from the resistant B6 strain also confers increased epilepsy susceptibility, suggesting that *Szs3* and *Moe1* may reflect the effect of the same B6 allele.

The epilepsy susceptibility locus *Moe2* on Chromosome 19 was confirmed by using a congenic mouse strain with this interval derived from strain SJL/J. We used the B6.*dblr* mouse strain with an SJL-derived interval on Chromosome 19. This provided a useful resource for confirmation of the *Moe2* locus, circumventing the need to generate an interval-specific congenic strain. Compared with littermates without the SJL-derived Chromosome 19 interval, (B6.Q54, *dblr*/+) mice had increased seizure incidence. The mutation in heterozygous *dblr*/+ mice results in a 20% reduction in expression of the Wnt inhibitor Dkk1 (MacDonald et al. 2004). As a control for the effect of reduced Dkk1 expression, a heterozygous null allele of Dkk1 with a 50% reduction in Dkk1 expression did not influence the epilepsy phenotype of *Scn2a*^{Q54} mice. This supports the hypothesis that the increased epilepsy susceptibility is a result of the SJL-derived interval of Chromosome

19. This region of Chromosome 19 has not been associated with seizure susceptibility or severity in previous QTL studies and represents a novel epilepsy susceptibility locus.

The 1-LOD support interval of the *Moe1* locus on Chromosome 11 contains more than 500 known and predicted genes and the *Moe2* locus contains more than 150 known and predicted genes. Likely candidate genes include ion channels, receptors, neurotransmitters and neuromodulators, transcription factors, and genes involved in neural development and injury response. Polymorphisms that result in changes in protein sequence or variants in regulatory elements are likely to underlie the genetic difference in seizure susceptibility between strains. Large-scale copy number polymorphisms are another type of variation whose role in genetic diversity is just beginning to be appreciated (Sebat et al. 2004). We screened the coding sequence of a small number of candidate genes but did not identify any coding polymorphisms.

More than 150 mutations in voltage-gated sodium channels have been identified in patients with epilepsy. Variable expressivity is a common feature of inherited epilepsy caused by sodium channel mutations because family members with the same mutation exhibit differences in the clinical severity of epilepsy. For example, in one GEFS+ family carrying the K1270T mutation in *SCN1A*, nine family members had isolated febrile seizures and five family members have TLE (Abou-Khalil et al. 2001). In another family with the T875M mutation in *SCN1A*, half of the family members had isolated febrile seizures while the other half had more severe seizure types including generalized tonic-clonic, absence, atonic, and hemicorporeal seizures (Moulard et al. 1999; Escayg et al. 2000). This variable expressivity suggests that other factors besides the primary sodium channel mutation influence the clinical manifestation of epilepsy in human patients. This could include stochastic events during development, accumulation of somatic mutations (Weiss 2005), environmental insults such as trauma, and differences in inheritance of genetic susceptibility alleles such as these mapped in the current study. Isolation of susceptibility genes and analysis of the underlying pathophysiology will contribute to understanding the molecular events of epileptogenesis and identify novel therapeutic targets.

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