

A POTASSIUM CHANNEL IS ASSOCIATED WITH RESISTANCE TO EPILEPSY

Fine Mapping of a Seizure Susceptibility Locus on Mouse Chromosome 1: Nomination of *Kcnj10* as a Causative Gene

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Previous quantitative trait loci (QTL) mapping studies document that the distal region of mouse chromosome (Chr) 1 contains a gene(s) that is in large part responsible for the difference in seizure susceptibility between C57BL/6 (B6) (relatively seizure-resistant) and DBA/2 (D2) (relatively seizure-sensitive) mice. We now confirm this seizure-related QTL (*Szs1*) using reciprocal, interval-specific congenic strains and map it to a 6.6-Mb segment between *Pbx1* and *D1Mit150*. Haplotype conservation between strains within this segment suggests that *Szs1* may be localized more precisely to a 4.1-Mb critical interval between *Fcgr3* and *D1Mit150*. We compared the coding region sequences of candidate genes between B6 and D2 mice using RT-PCR, amplification from genomic DNA, and database searching and discovered 12 brain-expressed genes with SNPs that predict a protein amino acid variation. Of these, the most compelling seizure-susceptibility candidate is *Kcnj10*. A survey of the *Kcnj10* SNP among other inbred mouse strains revealed a significant effect on seizure sensitivity such that most strains possessing a haplotype containing the B6 variant of *Kcnj10* have higher seizure thresholds than those strains possessing the D2 variant. The unique role of inward-rectifying potassium ion channels in membrane physiology coupled with previous strong association between ion channel gene mutations and seizure phenotypes puts even greater focus on *Kcnj10* in the present model. In summary, we confirmed a seizure-related QTL of large effect on mouse Chr 1 and mapped it to a finely delimited region. The

critical interval contains several candidate genes, one of which, *Kcnj10*, exhibits a potentially important polymorphism with regard to fundamental aspects of seizure susceptibility.

Association between Variation in the Human KCNJ10 Potassium Ion Channel Gene and Seizure Susceptibility

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PURPOSE: Our research program uses genetic linkage and association analysis to identify human seizure sensitivity and resistance alleles. Quantitative trait loci mapping in mice led to identification of genetic variation in the potassium ion channel gene *Kcnj10*, implicating it as a putative seizure-susceptibility gene. The purpose of this work was to translate these animal model data to a human genetic association study.

METHODS: We used single-stranded conformation polymorphism (SSCP) electrophoresis, DNA sequencing, and database searching (NCBI) to identify variation in the human *KCNJ10* gene. Restriction fragment length polymorphism (RFLP) analysis, SSCP, and Pyrosequencing were used to genotype a single-nucleotide polymorphism (SNP, dbSNP rs#1130183) in *KCNJ10* in epilepsy patients ($n = 407$) and unrelated controls ($n = 284$). The epilepsy group comprised patients with refractory mesial temporal lobe epilepsy ($n = 153$), childhood absence ($n = 84$), juvenile myoclonic ($n = 111$), and idiopathic generalized epilepsy not otherwise specified (IGE-NOS, $n = 59$), and all were of European ancestry.

RESULTS: SNP rs#1130183 (C>T) alters amino acid 271 (of 379) from an arginine to a cysteine (R271C). The C allele (Arg) is common, with conversion to the T allele (Cys) occurring twice as often in controls compared with epilepsy patients. Contingency analysis documented a statistically significant association between seizure

resistance and allele frequency, Mantel–Haenszel $\chi^2 = 5.65$, $df = 1$; $P = 0.017$; odds ratio, 0.52; 95% CI, 0.33–0.82.

CONCLUSIONS: The T allele of SNP rs#1130183 is associated with seizure resistance when common forms of focal and generalized epilepsy are analyzed as a group. These data suggest that this missense variation in *KCNJ10* (or a nearby variation) is related to general seizure susceptibility in humans.

COMMENTARY

Two recent articles published by Buono et al. describe variation in a potassium channel gene associated with seizures. The researchers first identified a polymorphism in the *Kcnj10* potassium channel gene that is associated with seizures in mice. After this discovery, they screened more than 400 epilepsy patients for sequence variation in the same gene. They found a similar polymorphism in the human *KCNJ10* gene that was associated with resistance to both generalized and partial epilepsy. This finding is remarkable and demonstrates that uncovering genetic determinants of seizure susceptibility in mice can lead to the identification of genes relevant to human epilepsy.

The researchers first found genetic variation in the *Kcnj10* gene by using the mouse as a model for epilepsy. The epilepsy trait in mice was measured by determining the electrical current required to induce a seizure, that is, the maximal electroshock seizure threshold (MEST) test. MEST testing has been widely used as a model for epilepsy. C57BL6 mice are relatively resistant and therefore have high seizure thresholds, whereas DBA2 mice are seizure sensitive and have low seizure thresholds. These two inbred mouse strains with differing thresholds to electroconvulsive shock were crossed, and the *Szs1* locus was mapped to chromosome 1 by using quantitative trait loci (QTL) analysis (1). The *Szs1* locus accounts for a large proportion of the difference in seizure susceptibility between C57BL6 and DBA2 mice and also is associated with increased susceptibility to kainic acid–induced seizures (2) and pentylenetetrazol-induced seizures (3).

Specific genes responsible for QTL are notoriously difficult to pinpoint. In part, the problem is that most quantitative traits are due to many genes of small effect, and epilepsy is no different. Another difficulty is that the QTL region is often very large. In the present report, Ferraro et al. used congenic strains (C57BL6 background with a DBA2 chromosome 1 or vice versa) with overlapping portions of chromosome 1 to refine the *Szs1* locus. MEST testing of these congenic strains refined the *Szs1* locus to a 4.1-megabase interval, containing approximately 100 genes. Four genes in this region were particularly interest-

ing to the investigators because they are directly involved in ion transport; the findings included two potassium channel genes, *Kcnj9* and *Kcnj10*. With the full sequence of both C57BL6 and DBA2 available, it was a relatively simple matter to identify all the single-nucleotide polymorphisms (SNPs) in the region that varied between the two strains and also changed an amino acid in the protein. The list was further narrowed to 12 genes by selecting only those genes that are expressed in the brain, and *Kcnj10* was still on the list. The possibility that one of the other 11 candidate genes identified is involved in seizure susceptibility cannot be ruled out.

The SNP identified in *Kcnj10* was examined in 15 different inbred mouse strains, and the researchers discovered that only the C57-related strains had a threonine at residue 262 of *Kcnj10*; all non-C57 mice had a serine. When these strains were tested by using the MEST test, strains with the threonine generally had higher seizure thresholds, that is, were resistant to seizures. The threonine was present in all species where sequence was available, except in non-C57-related mice. *Kcnj10* is a member of the inwardly rectifying potassium channel (K_{ir}) family and is an attractive epilepsy candidate; however, the SNP detected may not be responsible but merely tightly linked to another causative gene. No physiologic studies were presented, and it would be interesting to test K_{ir} current recordings from the different mouse strains to determine whether the threonine–serine substitution alters channel function.

The results provided convincing evidence for *Kcnj10* being involved in altered seizure threshold in mice. In their second article, Buono et al. describe variation in *KCNJ10* in patients with epilepsy. They found an arginine–cysteine variant at residue 271 of the protein, just nine amino acids away from the SNP described in mice. Cysteine is the less frequent allele and was present at a higher frequency in controls (7.9%) compared with epilepsy patients (4.2%), implying that the presence of the cysteine in the *KCNJ10* protein confers resistance to epilepsy. The P value ($P = 0.017$) for this association was statistically significant but must be confirmed in a separate population. The authors concluded that variation in *KCNJ10* is associated with multiple seizure types, rather than with any particular type of epilepsy. This seems to contrast with other reports, which demonstrated that genetic variation is associated with particular seizure disorders (4).

The results from the mouse and human studies provide strong evidence that the *KCNJ10* gene can confer resistance to epilepsy. In almost every published epilepsy family in which a discrete gene has been identified, several members of the family inherit the defective gene but do not have epilepsy. It would be interesting to test these nonpenetrant family members for the cysteine allele of *KCNJ10*, because this may explain why some people can inherit an epilepsy gene without developing seizures.

Potassium channels are the most diverse group of the ion channel family and are important both in shaping the action potential and in neuronal excitability and plasticity. The two main classes of potassium channels are voltage gated (K_v) and inwardly rectifying (K_{ir}) channels. Mutations in voltage-gated potassium channels have previously been associated with benign familial neonatal seizures (5). *KCNJ10* (the protein is also known as $K_{ir}4.1$) is an inwardly rectifying channel that helps regulate extracellular potassium ion concentrations. *KCNJ10* is expressed widely in the brain, predominantly by glial cells, with particularly high levels in the brainstem (6). Neurons become hyperexcitable when extracellular potassium levels are too high or too low (outside the 2- to 5-mM range). Potassium released from neurons is absorbed by astrocytes, through K_{ir} channels, and redistributed. Homozygous *Kcnj10* knockout mice die shortly after birth (7) and are probably not a good model for the more subtle genetic variation attributed to the seizure susceptibility. Both the mouse and human *KCNJ10* variants are within a region involved with ionic conductance, channel subunit dimerization, and anchoring to the plasma membrane (8). Any or all of these functions may be subtly affected by the amino acid substitutions present in the mouse and human potassium channels. Functional studies will help determine if the variants cause alteration of channel conductance.

Growing evidence exists that K_{ir} channels play an important role in epileptogenesis and may provide novel targets for the development of new antiepileptic drugs. Studies on the weaver mouse were the first to demonstrate that K_{ir} channels are involved in seizure generation (9). This mouse line has a point mutation in the pore region of the *Kcnj6* gene (also known as *Girk2* or *Kir3.2*). Studies in humans have so far failed to demonstrate association between *KCNJ6* and epilepsy (10). Knockout of *Kcnj11* (*Kir6.2*) also causes increased seizure susceptibility in mice (11). Polymorphisms in *KCNJ3* have been associated with epilepsy in humans (10), and reduced K_{ir} current has been reported in surgical tissue from patients with intractable temporal lobe epilepsy (11). The addition of *KCNJ10* to the list of K_{ir} channels associated with seizures emphasizes the importance of this gene family in epileptogenesis.

The two reports by Buono, Ferraro, and colleagues are excellent examples of QTL analysis in mice translating into identification of an epilepsy susceptibility gene in humans. It is an exciting prospect that a single gene may be associated with resistance to multiple seizure types and may aid in the development of new treatments. However, caution must be maintained until these results can be confirmed. Confirmation will require functional demonstration that the sequence variants have a physiologic effect, as well as the testing of an independent epilepsy population for the presence of the cysteine variant. With the growing molecular resources available for both mice and hu-

mans, this type of success story will become more common. In the future, when sequencing individual genomes becomes commonplace, we may be able to determine a person's epilepsy risk based on the presence of certain genetic variants, such as those found in *KCNJ10*. An individual's likelihood of developing epilepsy will probably be determined by a combination of many different susceptibility and resistance genes, in addition to environmental factors.

by Robyn Wallace, Ph.D.

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