New Ideas in Epilepsy Genetics

Novel Epilepsy Genes, Copy Number Alterations, and Gene Regulation

Christina A. Gurnett, MD, PhD; Peter Hedera, MD

The majority of genes associated with epilepsy syndromes to date are ion channel genes. Selection bias may have allowed us to establish their role in epilepsy based on a priori knowledge of the significance of these proteins in regulating neuronal excitability. There are, however, more than 3000 genes expressed at the synapse, as well as many other genes expressed nearby in supporting cells and glia that can likewise regulate excitability. Identification of new genes involved in epilepsy may arise from studying the targets of anticonvulsant medications, ascertainment of an epileptic phenotype in mice, or as a result of positional cloning efforts. There are several loci for idiopathic focal and generalized epilepsies that lie in chromosomal regions that are devoid of known ion channels; therefore, the number of novel genes involved in epilepsy is likely to increase. Establishing the role of these novel genes in the pathogenesis of epilepsy has not been an easy task compared with the relative ease with which ion channel mutations can be studied. This review will describe several novel epilepsy genes and will then discuss other genetic causes of epilepsy, including alterations of chromosomal copy number and gene regulatory elements.

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NOVEL EPILEPSY GENES

Leucine-Rich, Glioma Inactivated 1

At least 19 different leucine-rich, glioma inactivated 1 (LGI1) mutations have been described in families with autosomal dominant partial epilepsy with auditory features, a rare familial form of lateral temporal lobe epilepsy associated with aphasia and partial seizures with auditory disturbances (Table).¹ The function of LGI1 was unknown until the recent discovery of an interaction of this protein with the potassium channel Kv1.1.² In that study, LGI1 appears to alter the inactivation kinetics mediated by the KvB1 subunit, an effect that is abolished by the human mutations. Both truncation and missense mutations have been described, and both appear to cause reduced levels of protein,³ although the subcellular localization of LGI1 is not currently clear. Senechal et al⁴ reported LGI1 to be a secreted protein based on expression studies in transfected cells, whereas the model proposed by Schulte et al² suggests that LGI1 is associated with Kv1.1 through an intracellular interaction. LGI1 was also recently demonstrated to be an extracellular ligand associated with a postsynaptic complex containing several proteins implicated in epilepsy, including an α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) regulatory protein (ADAM22) and stargazin.⁴ LGI1 appears to enhance AMPA-receptor neurotransmission, while mutated LGI1 has no
domain–containing protein (EFHC1) in 6 unrelated
associated with missense mutations in the EF-hand
Familial juvenile myoclonic epilepsy (JME) has been
with the
nearby genes are responsible for epilepsy associated
with hearing loss and retinitis pigmentosa, but not epilepsy.
There is also no evidence of epilepsy in carriers of
drome type 2, a condition that results in congenital
splicing variants. Thus, it is possible that alterations of
heterozygous mice. Furthermore, homozygous muta-
tions of
recessive manner with no evidence of epilepsy in the
The role of MASS1/VLGR1 in human epilepsy is not
entirely clear. The MASS1/VLGR1 gene resides within a
locus for febrile seizures, FEB4; however, mutations in
MASS1/VLGR1 have not been identified in the families
showing linkage to this region. Although heterozygous
mutations have been described in 2 small Japanese
families with febrile and afebrile seizures,7 audiogenic-
induced epilepsy in mice is inherited in an autosomal-
recessive manner with no evidence of epilepsy in the
heterozygous mice. Furthermore, homozygous muta-
tions of MASS1/VLGR1 in humans cause Usher syn-
drome type 2, a condition that results in congenital
hearing loss and retinitis pigmentosa, but not epilepsy.
There is also no evidence of epilepsy in carriers of
Usher syndrome mutations; MASS1/VLGR1 is a compli-
cated gene, encoded by more than 90 exons spanning
nearly 600 kilobases. There is the possibility of addi-
tional complexity contributed by extensive alternative
splice variants. Thus, it is possible that alterations of
MASS1/VLGR1 regulatory regions or mutations in other
nearby genes are responsible for epilepsy associated
with the FEB4 locus.

**EF-Hand Domain–Containing Protein**

Familial juvenile myoclonic epilepsy (JME) has been
associated with missense mutations in the EF-hand
domain–containing protein (EFHC1) in 6 unrelated
families with JME. When overexpressed in hippocam-
pal neurons, EFHC1 induces apoptosis, an effect that is
abolished by mutations in EFHC1. The apoptotic effect of
EFHC1 appears to result from an augmentation of an
R-type calcium current resulting from a direct interac-
tion of EFHC1 with calcium channel Cav2.3. It was
thus proposed that reduced apoptosis causes subtle
developmental cortical abnormalities that eventually
result in epilepsy. Replication studies have failed to

<p>| Table. Susceptibility Genes for Human Epilepsy |
|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th><strong>Genes</strong></th>
<th><strong>Disorder</strong></th>
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<tr>
<td>Novel genes</td>
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<tr>
<td>LG1</td>
<td>Autosomal dominant partial epilepsy with auditory features</td>
<td>10q24</td>
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<tr>
<td>MASS1/VLGR1</td>
<td>Febrile and afebrile seizures</td>
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<td>EFHC1</td>
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<td>6p21.3</td>
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<td>ME2</td>
<td>Idiopathic generalized epilepsy</td>
<td>18q21</td>
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<td>IMPA2</td>
<td>Febrile seizures</td>
<td>18p11</td>
</tr>
<tr>
<td>CNTNAP2</td>
<td>Focal epilepsy</td>
<td>7q36</td>
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<td><strong>Sodium channel</strong></td>
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<td>SCN2A</td>
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<tr>
<td><strong>Calcium channel</strong></td>
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<td>CACNA1A</td>
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<td>CACNA1H</td>
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<td>CACNB4</td>
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<td><strong>Potassium channel</strong></td>
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<td><strong>Acetylcholine receptor</strong></td>
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<tr>
<td>GABRD</td>
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Abbreviations: GABA, y-aminobutyric acid; GEFS+, generalized epilepsy and febrile seizures plus locus.
identify mutations in the majority of familial and sporadic JME (P.H., unpublished data, 2006), providing further evidence that mutations in EFHC1 represent the minority of patients with JME. A tentative association was also recently made with JME and a related gene, EFHC2.

MALIC ENZYME 2

Malic enzyme 2 (ME2) is a mitochondrial enzyme expressed in the brain that converts malate to pyruvate, thus providing substrate for the synthesis of γ-aminobutyric acid. Single nucleotide polymorphism haplotypes centered on ME2 were recently shown to be associated with adolescent-onset idiopathic generalized epilepsy, thus confirming previous linkage data implicating a locus on chromosome 18 in idiopathic generalized epilepsy. Although none of the ME2 single nucleotide polymorphisms were reported to alter the function of the enzyme through currently known mechanisms, the analysis supported an autosomal recessive pattern of inheritance that is consistent with other disorders resulting from enzymatic activity. The association of ME2 with idiopathic generalized epilepsy was unable to be replicated in another large cohort, suggesting that the epileptogenic effect may be small or seen only in selected populations. The plausibility of a metabolic enzyme being involved in generalized epilepsy is compelling and novel.

BROMODOMAIN-CONTAINING PROTEIN 2

Chromosome 6p has long held promise as a region containing a gene(s) for idiopathic generalized epilepsy. In a subset of families showing linkage to chromosome 6, positive associations were found with JME and single nucleotide polymorphisms in bromodomain-containing protein (BRD2). Sequencing of BRD2 in these individuals did not reveal a causative mutation but did uncover several promoter variants of uncertain significance; BRD2 is a bromodomomain protein that may control transcription through direct interactions with acetylated histones. It was proposed that alteration of BRD2 function during development disrupts normal neuronal connectivity and causes epilepsy.

MYOINOSITOL MONOPHOSPHATASE

Despite the identification of multiple susceptibility loci for febrile seizures, identifying responsible genes has not been easy in most cases. Nakayama et al reported linkage and association of febrile seizures to the IMPA2 gene on chromosome 18 in a cohort of Japanese nuclear families. IMPA2 encodes myoinositol monophosphatase, which is an enzyme in the phosphatidylinositol signaling pathway. A potential role for myoinositol monophosphatase in the causality of epilepsy is strengthened by the knowledge that its activity is inhibited by lithium (a proconvulsant) and stimulated by carbamazepine (an anticonvulsant). However, causative gene mutations, resulting in known effects on function or expression, have not been reported.

CONTACTIN-ASSOCIATED PROTEIN-LIKE 2

A recent report described a homozygous mutation in contactin-associated protein-like 2 (CNTNAP2) in Amish children with intractable, focal epilepsy, developmental regression, and cortical dysplasia. The protein encoded by CNTNAP2 clusters voltage-gated potassium channels ( Kv1.1) at the nodes of Ranvier. Surgically resected brain tissue from affected individuals shows altered expression of Kv1.1 and Nav1.1 channels, which may contribute to the pathogenesis of disease. Some affected individuals also manifest radiographically evident cortical malformations, suggesting the possibility that alterations of both ion channel function and cortical development result from CNTNAP2 mutations.

CHROMOSOMAL COPY NUMBER ALTERATIONS

Comparative genomic hybridization has revealed an additional level of genomic complexity through the identification of relatively common deletions and insertions throughout the genome in all individuals, some of which may be associated with disease. These copy number changes may be below the resolution of standard G-banded karyotype that typically can distinguish only genomic alterations greater than 5 to 10 megabases (Figure, A). Depending on the array platform, deletions and insertions can now be resolved at 5000 or fewer base pairs (Figure, B). Current clinical applications for array comparative genomic hybridization include the evaluation of several hundred known regions of human variation of significance in disease (eg, Wolf-Hirschhorn syndrome [chromosome 4p−], Angelman syndrome, and DiGeorge Syndrome). Array comparative genomic hybridization studies may eventually replace the karyotype in the initial workup of individuals with dysmorphic features, malformations, or developmental delay. However, structural alterations of the genome, including balanced translocations, inversions, and point mutations, are not typically detected by array comparative genomic hybridization.

Research studies in epilepsy are likely to utilize genome-wide arrays with better resolution than those currently used clinically. Sherr et al evaluated 25 individuals with agenesis of the corpus callosum with array comparative genomic hybridization using 2464 elements at an approximate resolution of 1.4 megabases; they identified 3 de novo copy number changes. Because of the high frequency of copy number variants in healthy individuals, the inclusion of parental controls is required, particularly until common normal variants can be cataloged. Copy number variants may contain entire genes or exons of individual genes and may alter non-gene regulatory regions, adding to the many possible mechanisms of disease pathogenesis. A handful of human diseases are reported to result from copy number variations, but the number is expected to increase.

GENE REGULATION BY LONG-RANGE ENHANCERS

Comparison of genomic sequences across diverse species has demonstrated that the majority of highly conserved sequences do not encode genes but consist of elements that
are interspersed between genes, often in gene deserts or hidden within introns of genes. The functions of many of these nongene elements are not known, but some appear to act as tissue-specific enhancers of developmentally expressed genes that can act from as far away as 1 megabase. Disruption of the brain-specific enhancer sequence lying several hundred kilobases proximal to the sonic hedgehog gene can result in holoprosencephaly, a phenotype nearly identical to that produced by mutations within the sonic hedgehog gene itself.\textsuperscript{16} Recently, epilepsy in patients with X-linked recessive hypoparathyroidism was shown to result from an interstitial deletion-insertion downstream of the SOX3 gene that likely disrupts a distant regulatory sequence.\textsuperscript{17} Because many of these long-range enhancers act on developmentally expressed genes or genes involved in signaling, it is possible that their role in epilepsy may be limited to developmental epilepsies.

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Figure. Comparison of techniques used to determine chromosomal alterations related to epilepsy. A, Chromosomal karyotype revealed the presence of additional material of an unknown source attached to the long arm of chromosome 3 in a patient with epilepsy and developmental delay. B, A genome-wide array of comparative genomic hybridization revealed that the additional material was from chromosome 8, which showed a clearly defined duplication of distal chromosome 8, as well as multiple other smaller deletions (C.A.G., unpublished data, 2006).
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REFERENCES


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