Susceptibility genes for complex epilepsy

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Common idiopathic epilepsies are, clinically and genetically, a heterogeneous group of complex seizure disorders. Seizures arise from periodic neuronal hyperexcitability of unknown cause. The genetic component is mostly polygenic, where each susceptibility gene in any given individual is likely to represent a small component of the total heritability. Two susceptibility genes have been so far identified, where genetic variation is associated with experimentally demonstrated changes in ion channel properties, consistent with seizure susceptibility. Rare variants and a polymorphic allele of the T-type calcium channel \textit{CACNA1H} and a polymorphic allele and a rare variant of the GABAA receptor \textdelta subunit gene have differential functional effects. We speculate that these and other as yet undiscovered susceptibility genes for complex epilepsy could act as ‘modifier’ loci, affecting penetrance and expressivity of the mutations of large effect in those ‘monogenic’ epilepsies with simple inheritance that segregate through large families. Discovery of epilepsy-associated ion channel defects in these rare families has opened the door to the discovery of the first two susceptibility genes in epilepsies with complex genetics. The susceptibility genes so far detected are not commonly involved in complex epilepsy suggesting the likelihood of considerable underlying polygenic heterogeneity.

INTRODUCTION

Idiopathic epilepsy is regarded as primarily genetic, and complex generalized epilepsies (IGEs), the subject of this review, represent at least half of the estimated 70% of epilepsies that are idiopathic (1). Complex epilepsies are polygenic, as suggested by rapidly diminishing risks beyond first-degree relatives (2) and high concordance between monozygotic twins (3). The IGEs consist of various subsyndromes. These include the classical IGEs: childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy (JME) and a more recently recognized group, referred to as generalized epilepsy with febrile seizures plus (GEFS\textsuperscript{+}).

Over the past decade, a number of genes have been associated with the rare monogenic idiopathic epilepsies that have relatively simple inheritance (Table 1), revealing the importance of ion channel genes in epilepsy. Pedigrees of families with monogenic epilepsy have been found to segregate various ion channel mutations of large effect. Although the molecular-based classification of monogenic idiopathic generalized and partial epilepsy is now well underway, progress towards discovering the underlying genetic variation for susceptibility to complex idiopathic epilepsy has only just begun, with the recent identification of two susceptibility genes affecting transport of ions into the neuron (Table 1). Because the majority of genes identified for ‘monogenic epilepsy’ encode ion channel subunits, those genes encoding or regulating neuronal ion channels were the initial prime candidates to be tested as susceptibility genes for complex epilepsy.

Complex epilepsy arises when, by chance, meiotic reshuffling creates a combination of susceptibility alleles with sufficient effect in the same individual to push neuronal hyperexcitability over the seizure threshold. Each susceptibility allele alone is insufficient to cause seizures, but requires the additive or epistatic interaction of other susceptibility alleles. Complex epilepsy is most often expressed sporadically in an affected individual, and the phenotype does not follow
Table 1. Genes and susceptibility loci for ‘monogenic’ and complex idiopathic epilepsies (35–41,52–54)

<table>
<thead>
<tr>
<th>Epilepsy gene</th>
<th>Epilepsy syndrome</th>
<th>Year of discovery</th>
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<tbody>
<tr>
<td><strong>Monogenic channelopathies</strong></td>
<td></td>
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<tr>
<td>CHRNA4</td>
<td>ADNFLE</td>
<td>1995</td>
</tr>
<tr>
<td>KCNQ2</td>
<td>BFNS</td>
<td>1998</td>
</tr>
<tr>
<td>SCN1B</td>
<td>GEFS⁺</td>
<td>1998</td>
</tr>
<tr>
<td>SCN1A</td>
<td>GEFS⁺/(SMEI)</td>
<td>2000/(01)</td>
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<tr>
<td>CHRNB2</td>
<td>ADNFLE</td>
<td>2000</td>
</tr>
<tr>
<td>GABRG2</td>
<td>CÆ/FS/GEFS⁺</td>
<td>2001</td>
</tr>
<tr>
<td>SCN2A</td>
<td>GEFS⁺/BFNIS</td>
<td>2001/02</td>
</tr>
<tr>
<td>GABRA1</td>
<td>ADME</td>
<td>2002</td>
</tr>
<tr>
<td>CLCN2</td>
<td>IGE</td>
<td>2003</td>
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<tr>
<td><strong>Complex epilepsies: channelopathies</strong></td>
<td></td>
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<tr>
<td>CACNA1H</td>
<td>CAE, IGE</td>
<td>2003/04</td>
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<tr>
<td>GabRD</td>
<td>IGE, GEFS⁺</td>
<td>2004</td>
</tr>
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Abbreviations in order of appearance: Genes: CHRNA4: acetylcholine receptor subunit, α4; KCNQ2/KCNQ3: potassium channel subunits; SCN1B: sodium channel β1 subunit; SCN1A: sodium channel α1 subunit; CHRN2: acetylcholine β2 receptor subunit; GABRG2: GABBARα3 receptor γ2 subunit; SCN2A: sodium channel α2 subunit; GABRA1: GABBARα receptor α1 subunit; CLCN2: chloride channel gene 2; LGI1: leucine-rich, glioma inactivated 1 gene; EFHC1: EF-hand motif gene; CACNA1H: T-type calcium channel; GABRD: GABBARα receptor δ subunit;.

**Syndromes:** ADNFLE: autosomal dominant nocturnal frontal lobe epilepsy; BFNS: benign familial neonatal seizures; GEFS⁺: generalised epilepsy with febrile seizures plus; SMEI: severe myoclonic epilepsy of infancy; CAE: childhood absence epilepsy; FS: febrile seizures; BFNIS: benign familial neonatal-infantile seizures; ADJME: autosomal dominant juvenile myoclonic epilepsy; IGE: idiopathic generalised epilepsy; ADPEAF: autosomal dominant partial epilepsy with auditory features; JME: juvenile myoclonic epilepsy.

Mendelian inheritance. Nor do the close relatives, who may or may not be affected, necessarily share the same set of epilepsy susceptibility alleles. During intergeneration transmission, each susceptibility allele is segregated away from other unlinked susceptibility alleles.

**APPROACHES TO DETECTING SUSCEPTIBILITY GENES FOR COMPLEX EPILEPSY**

Gene mutations in large families with monogenic epilepsy have been identified by linkage mapping and candidate gene analysis. Linkage mapping applied to complex epilepsy has suggested various susceptibility regions (4–13), but with a poor record of replication. Studies have used both parametric (in multiplex IGE families) and non-parametric linkage approaches (sib pair, affected pedigree member). None has led to the identification of a ‘common epilepsy gene’, reinforcing the concern that complex epilepsy syndromes, as presently defined by clinical examination alone, may be too genetically heterogeneous for the proposed SNP-based disease association approach to succeed.

Non-replicable (14) case–control candidate gene (genome-wide or hypothesis-driven candidate) or transmission disequilibrium tests of disease association (where associations with either a functional SNP or its haplotype block are detected) have not yet led to any genes for complex epilepsy. Association testing has generally been underpowered to detect any small effects of polygenes (14) and has assumed patient homogeneity (identical phenotype, homogeneity of underlying polygenic variants, absence of population stratification). Genetic associations with complex diseases in general (15) and epilepsy in particular (14) have a poor record of replication either because underlying assumptions, such as a homogeneous test population, are incorrect, or the associations found are spurious statistical artefacts of multiple testing.

Studies using ion channel electrophysiology proved crucial as the monogenic channelopathy story for epilepsy unfolded. Putative ion channel mutations of large effect in families are accepted as causative, when measurable and statistically significant perturbation of channel properties are found and can be related to an epilepsy-causing mechanism. These channel changes either increase or decrease the flow of ions (calcium, sodium, potassium, chloride) into or out of the neuron, thus altering the excitation/inhibition balance (16–23). For complex epilepsy, similar effects are predicted, but the effects will be more subtle, such that the additive or epistatic effects of more than one susceptibility gene within the same individual will be required to precipitate an epilepsy phenotype. To date, ion channel electrophysiology of SNPs and rare variants have been the most definitive approach to the identification of susceptibility genes for complex epilepsy, but only small-scale studies have been attempted so far and only for epilepsies that might be complex channelopathies.

Statistical prospecting through genome-wide SNP association studies might become frequently utilized for complex disorders including epilepsy, to identify the regions of the genome containing susceptibility genes of significant effect, irrespective of whether or not they are ion channels. The success of this approach will rely on the validity of the ancestral common variant complex epilepsy model (ACVCE), an epilepsy-specific version of the general common disease common variant model (24–30). The numbers of SNPs that can potentially function as susceptibility alleles for complex disorders are almost unlimited, with millions now documented in public databases (31). Association studies will only work under the ACVCE model if there is sufficient patient homogeneity to avoid undermining the power of the statistical association.

Rare variant loci in epilepsy relate to the alternative multiple rare variant complex epilepsy model (MRVCE), an epilepsy-specific version of the general common disease rare variant model (28,32,33). We may carry as many as 500–1200 slightly deleterious mutations (34), although many may only have a detectable effect on phenotype as two deleterious copies (i.e. recessive). Association studies will not work for complex epilepsy or any other complex disorder if the pathogenic variation fits only the MRVCE model. Intuitively, genetic variation fitting either model could account for complex epilepsy, and sets of susceptibility alleles applicable to any of the complex epilepsies could be drawn from both models.
CHARACTERIZATION OF CACNA1H AND GABRD AS THE FIRST SUSCEPTIBILITY GENES FOR COMPLEX EPILEPSY

Both susceptibility genes for complex epilepsy have been identified by candidate gene screening and subsequent electrophysiological validation. These functional ion channel genes, among many other ion channel gene candidates, were tested due to their relationship with genes previously implicated in monogenic epilepsy, using the same process of electrophysiological validation.

Variants in the T-type calcium channel CACNA1H, involved in regulation of neuronal firing, were associated with CAE (35), and studies were extended to include other IGEs (36). Location of the amino acid variants in evolutionarily conserved sequences strongly suggested a functional role. The CACNA1H variants did not segregate in large families, so it required electrophysiological analysis to confirm their susceptibility role in complex epilepsy. Three CAE-associated variants, F161L, E282K and V831M, and two IGE-associated variants, P618L and G755D, exhibited altered channel properties consistent with increased propensity for seizures (37,38). Subsequently, the polymorphic SNP R788C was found to have a functional effect (39). Interestingly, the effect for R788C combined with the CAE-related G773D variant was different from the properties displayed by either sequence separately. Thus, in addition to additive effects, epistatic interactions between variants are also likely to play a significant role in complex epilepsy and complex disorders in general.

The GABAA receptor is the primary mediator of synaptic inhibition in the brain, and the subunits encoded by GABRG2 and GABRA1 are associated with monogenic forms of IGE and GEFS+ (Table 1). The gene encoding the δ-subunit of the GABAA receptor, GABRD, is a susceptibility gene for complex IGE based on the electrophysiological effects of ion channels containing the E177A and R220H variants (40,41). The δ-subunit is present in extra- and perisynaptic receptors which mediate tonic inhibition, suggesting that this mechanism plays a role in epilepsy. The rare E177A variant was present in one small GEFS+ family, and the low-frequency polymorphism R220H was present at a similar frequency in patients and controls, suggesting that GABRD is rarely implicated in complex epilepsy. These early data suggest that the polygenic spectrum underlying complex epilepsy will be as genetically heterogeneous as the genetic variation underlying monogenic epilepsy, because few cases of sporadic IGE carried the polymorphic variant.

These early studies involving the genetic variation underlying complex epilepsies illustrate the crucial role that ion channel electrophysiology is also playing in the identification and validation of susceptibility loci. Effects of each susceptibility allele will be more subtle than changes associated with monogenic epilepsy. The additive or interactive effects of multiple susceptibility alleles within the unique genome of an affected individual may result in an indistinguishable phenotype from that associated with a unique combination of other susceptibility genes in another individual, and these epilepsy phenotypes may even be indistinguishable from phenotypes associated with mutations of large effect in monogenic epilepsy. So far, most CACNA1H and one GABRD susceptibility variant are consistent with the MRVCE model. The susceptibility polymorphisms of GABRD and CACNA1H are consistent with the ACVCE model. Thus, a mixture of both models underlies complex epilepsy. The GABRD polymorphic susceptibility allele was no more prevalent among affected cases than controls, so was recognized as being undetectable by the association study approach (40). This was subsequently confirmed in another population (42).

INFERENCES NOW POSSIBLE FOR GENE AND ALLELIC ARCHITECTURE OF COMPLEX EPILEPSY

It has taken a decade from the discovery of the first monogenic epilepsy gene (43) until demonstration of the first two susceptibility genes for complex epilepsy (35–40). The rate of discovery of genes for monogenic epilepsy is decreasing because of the rarity of large families and minimal clues for discrimination of good epilepsy gene candidates among all brain expressed genes, other than genes that encode ion channels. The variation in expression and penetrance within large families segregating the so-called monogenic epilepsies strongly suggest that modifier genes (equivalent to susceptibility genes for complex epilepsies) act with the mutated gene in moulding the final phenotype. The syndromes similar to those seen in monogenic epilepsy may arise instead from the combined effects of unknown numbers of susceptibility genes. Thus, monogenic epilepsy may be regarded as an extreme case of the MRVCE model of complex polygenic epilepsy with the effect size of one of the strongest ‘susceptibility’ genes dominating the remainder of the genes involved, such that it segregates with epilepsy through a large family. Epilepsies may be categorized into three distinctive architectures: complex epilepsies, monogenic epilepsies and those epilepsies that are a component of the phenotypic spectrum for other Mendelian syndromes (Fig. 1).

The MRVCE model is clearly responsible for some of the heritability for complex epilepsy. The ACVCE model is responsible for the remainder, but there are two forms of this model. What we term the simplistic ACVCE model (Fig. 2A) is the only model where polymorphic susceptibility alleles will be detected by the association study approach. However, both of the polymorphic susceptibility alleles so far characterized (of CACNA1H and GABRD) fit what we term the realistic ACVCE model (Fig. 2B), named as such because this is the model which so far fits the limited amount of data available. Figure 2C incorporates the example of the polymorphic GABRD susceptibility allele into the realistic model. The proposed genetic architecture for complex epilepsy is a subset of susceptibility alleles drawn from a much larger pool of susceptibility alleles, rare or polymorphic. Thus, each of the polymorphic susceptibility alleles will not be widely represented among affected cases, and at variance with the simplistic model.

The validated involvement, but rarity of involvement of specific susceptibility genes such as CACNA1H and GABRD as effectors in complex epilepsy, is consistent with the observations of similar extreme genetic heterogeneity in the monogenic epilepsies (Table 1). SCN1A, SCN1B and GABRG2 mutations of large effect account for few of the
GEFS\(^+\) cases, and CHRNA4 and CHRNB2 account for few of the nocturnal frontal lobe cases (44). Figure 3 demonstrates the extreme seizure heterogeneity that can be associated even with the same mutation within the same family with a monogenic epilepsy. Extrapolated to complex epilepsies,
with multiple underlying susceptibility genes, the genetic heterogeneity for any of the subsyndromes shown in the GEFS\(^+\) spectrum (Fig. 3) may be enormously multiplied, making the statistical task of teasing out associations extremely challenging.

**FUTURE**

There is currently very little information on the identity of genes for complex epilepsy. The basic questions that need to be answered are these: how many genes are involved in complex epilepsy? What is the magnitude of their effects? Will common variants, rare variants or both substantially underlie the common and complex epilepsies? Will this variation reside primarily in the coding DNA? To what extent can manipulation of the environment, including drug therapy acting on sufficient numbers of susceptibility loci, reduce the affected patients’ seizure propensity (below the seizure threshold)? Will additive and interacting environmental determinants be just as complex as the combinations of polygenic determinants in complex epilepsy? Will it be possible to eventually identify enough of the multiple genetic risk factors for complex IGE to combine them into a genetic risk profile for any given individual?

The genetic architecture of complex diseases is complex enough considering only the coding sequence variants. The extent to which allelic variation in gene regulation, such as ion channel subunit expression, is modulated by changes in conserved non-coding genomic DNA sequences (45), microsatellite variation near coding sequences, polymorphic segmental genome duplications (46–48) and potential products of their non-homologous recombination and variation in splicing of multi-exon genes (49) has just begun to be investigated. Recently, epigenetic changes have been explored (e.g. DNA methylation, chromatin remodelling) as an alternative to DNA sequence changes for affecting expression (50). There is also the physiological decline in mitochondrial function with age. Does this explain some of the susceptibility to late-onset epilepsies?

The final question will be whether validated susceptibility genes cross racial boundaries. Allele frequencies may differ between races, but intuitively, differential functional effects between SNP alleles is a property likely to be retained across ethnic boundaries, especially within similar environments. On the basis of existing data from validated associations from a range of complex disorders, functional properties do remain relatively consistent across racial boundaries (51), and this will probably turn out to be the case for complex epilepsy. This will mean universal application of drug developments for the treatment of common idiopathic epilepsies in humans.

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