

## **Genetics of large populations in epilepsy: Association studies – Trials and tribulations**

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### **Abstract**

Unlike Mendelian epilepsies, complex epilepsies may be due to the effect of several or multiple susceptibility genes. Association studies have been used in the past decade to help identify common genetic variants underpinning complex epilepsies. However, as the data in the Epilepsy Genetic Association Database (epiGAD) show, many of these studies have been negative, or have shown conflicting results. Overall, most putative gene-disease associations are weak. Inadequate sample size is the most likely cause. Multicentre collaborations are vital.

### **INTRODUCTION**

In order to find a gene for epilepsy, one must first understand if the epilepsy syndrome follows Mendelian or complex inheritance.<sup>1</sup> In the case of Mendelian epilepsies, linkage analysis is the tried-and-tested method. Linkage analysis starts with families with epilepsy, and basically works on the premise that the causative gene mutation must be located close to other genetic markers that track with the disease.

For complex inheritance (which encompasses most common epilepsies), linkage analysis does not work so well. This is because the disease itself is due to multiple genes with varying effect sizes. The association study approach is then used. Association studies are population-based case-control studies. They examine the frequency of a genetic variant in cases and controls. This variant is said to be associated if the frequency varies more than expected by chance alone. The type of genetic variant examined varies from study to study. Most studies examine single nucleotide polymorphisms (SNPs), but other variants such as indels, block substitutions, inversions, and copy-number variants (CNV) can also be studied.<sup>2</sup>

### **EPILEPSY GENETIC ASSOCIATION STUDIES**

Most epilepsy association studies have studied SNPs, under the assumption that common variants (eg. SNPs with a mean allele frequency of >1%) underpin the genetic bases of complex epilepsies.<sup>1</sup> The typical approach is to pick a candidate gene (such as an ion channel or neurotransmitter receptor gene), select SNPs within the gene as

markers, assemble a group of cases and controls, then examine the SNP frequencies in cases and controls. One newer approach is the genome-wide association study (GWAS)<sup>3</sup>, where instead of selecting one gene, one searches for differences in variant frequencies across 500,000 to 1 million markers across multiple genes over the entire genome.

So what is the track record of such association studies? We have studied just over 200 such studies<sup>4</sup>, which have been databased online at the Epilepsy Genetic Association Database (epiGAD, [www.epigad.org](http://www.epigad.org)). Almost all were candidate gene association studies, and the majority examined SNPs. The majority (80%) examined epilepsy susceptibility genes, while the remainder analysed genes influencing epilepsy pharmacogenetics.

We set out to examine just how strong the evidence for positive associations were, using published criteria for assessing strength and credibility of genetic association studies.<sup>5</sup> We also analysed sample sizes. Using the published criteria, we found<sup>4</sup> that no gene-disease association in epilepsy qualified as 'strong'. Several gene-disease associations, such as the association of HLA-B\*1502 and hypersensitivity to carbamazepine, qualified as 'moderate'. The majority of associations qualified as 'weak'.

The most likely reason for the weak associations was sample size – 80% of studies recruited <200 cases. The good news was that sample sizes were gradually increasing. Studies published from 2005-2008 had a mean sample size of 202 cases, while studies published from 1996-2004 only had a mean case sample size of 118. The bad news however is that sample sizes remain

disconcertingly small, on an absolute level. This implies that the power to detect common variants with small effect sizes (odds ratio < 1.5) remains low.<sup>6</sup> A recent negative GWAS in focal epilepsy<sup>7</sup> soberingly reminds us even with large sample sizes of >3,000 cases and almost 7,000 controls, variants with small effect sizes (odds ratio <1.3) will still be missed.

### RARE VARIANTS

The landscape however for epilepsy genetic association studies changed in 2009, where rare variants were found to be associated with idiopathic generalized epilepsies.<sup>8</sup> These rare variants, which are found in <1% of the general population, influence epilepsy susceptibility. As these are rare variants, the effect size is typically larger (odds ratios >5), and genetic association studies examining rare variants may potentially identify these variants with sample sizes of about 330 cases and 330 controls<sup>6</sup>, if these rare variants are grouped. In order to do so, multicentre collaboration is imperative.

### WHERE TO FROM HERE?

Some have touted genetic meta-analysis as a way to resolve the many discordant findings of positive and negative studies in epilepsy. While some progress has been made<sup>9</sup>, methodological weaknesses in many of the primary studies makes meta-analysis difficult. Between-study heterogeneity, especially in phenotype definition, remains another major problem.

Realistically, no matter whether we pursue candidate-gene association studies or GWAS, multicentre collaborations will be needed to achieve requisite sample sizes. As the costs of sequencing fall, deep resequencing may emerge as an alternative to association studies.<sup>7</sup> As we gradually determine the genetic architecture of common epilepsies in the next few years, our new understanding will guide us in deciding the appropriate methodology.

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