

SCIENTIFIC COMMENTARY

Copy number variants—an unexpected risk factor for the idiopathic generalized epilepsies

Idiopathic generalized epilepsies account for 30% of all epilepsies and are the most common group of inherited seizures. They follow complex inheritance patterns involving multiple genes and environmental factors. The idiopathic generalized epilepsies are the most challenging and perhaps the most important area of epilepsy in which further genetic analysis is required; and understanding genetic determinants of the idiopathic generalized epilepsies will be the key to unravelling the neurobiology and suggesting improved therapies for these disorders.

The broad field of complex genetics now entertains two non-exclusive models for the genetic architecture of common diseases. The 'common disease, common variant' model posits that combinations of genetic variants frequent in the population cause the disease in question. This model is currently best tested by genome-wide association studies where 500 000 or more single nucleotide polymorphisms are simultaneously examined in large cohorts. Recent studies have revealed numerous common variants for many diseases but the effect size of each is typically very low (relative risks 1.1–1.5). In general, common variants do not appear to explain the major part of the attributable genetic risk for most complex diseases. These studies have, however, revealed pathways implicated in some common diseases. For the idiopathic generalized epilepsies, no common variants have been discovered; however, one explanation may be that appropriately powered studies have not yet been completed.

The second model is the 'common disease, rare variant' model. Here, multiple rare variants combine to cause disease and the model is tested by complete re-sequencing of the relevant genes in cases and controls. In the idiopathic generalized epilepsies, there is now some evidence supporting the multiple rare variant model based on the finding of mutations in genes such as the calcium channel subunit gene *CACNA1H* and the microtubule-associated protein *EFHC1*, the precise function of which is uncertain (Chen *et al.*, 2003; Suzuki *et al.*, 2004).

Until recently our concept of the human genome was of 23 pairs of chromosomes, each with a continuous, folded strand of the elegant double helix that remains unchanged in gene number from generation to generation. Now we know that deletion or duplication of various stretches of DNA, usually incorporating a number of genes, occur frequently throughout the genome in healthy subjects. These copy number variants result in the usual

two copies of a gene changing to a single copy in a heterozygous deletion or more than two copies in duplications. Surprisingly, these submicroscopic rearrangements often have no phenotypic expression, but they can act as rare variants predisposing to complex disease.

Copy number variants are invisible on routine karyotyping, but are readily revealed using higher definition molecular techniques such as single nucleotide polymorphism microarrays or array comparative genomic hybridization. These techniques allow at least 10 times the resolution of chromosomal structure and, as techniques are further refined to allow still greater resolution, the number of copy number variants will undoubtedly increase. The application of these techniques has permitted targeted and whole-genome approaches to genotyping of large disease populations and has already yielded impressive results.

Larger copy number variants are more likely to be associated with disease although size does not directly correlate with gene content. Some disease-causing copy number variants may contain a single gene whilst others have many genes deleted or duplicated. Often it is not clear which gene within the segment containing a particular copy number variant is responsible for the disease in question. One area in which this form of genetic susceptibility has been shown to be critically important is intellectual disability where large copy number variants explain the underlying aetiology in ~15% of individuals (Mefford and Eichler, 2009). Given the longstanding appreciation of cytogenetically visible chromosomal aberrations causing mental retardation, this observation is perhaps not surprising. Less expected is the observation that copy number variants play a role in conditions without cognitive impairment such as renal disease, thrombocytopenia and epilepsy.

Although there are many pathogenic non-recurrent copy number variants causing disease that are effectively 'private mutations', relevant to one or a few individuals, increasing attention has been paid to recurrent copy number variants. It is now well recognized that recurrent structural rearrangements occur at specific chromosomal loci. This is explained mechanistically by interspersed segmental duplications that sensitize ~10% of the genome to copy number variants because of unequal crossing over during meiosis (Mefford and Eichler, 2009). These rearrangements have a characteristic genomic architecture. They have a

length of unique sequence of 50 kb to 10 Mb flanked on each side by large, ≥ 10 kb, highly homologous segmental duplications that are effectively rearrangement 'hotspots'. There are >20 such recurrent copy number variants that result in >30 diseases (Mefford and Eichler, 2009).

A fascinating twist in the copy number variant tale is the discovery that phenotypic heterogeneity occurs with certain copy number variants. A good example is the 15q13.3 microdeletion now studied in many thousands of patients. It was first identified in 0.3% of patients with mental retardation, dysmorphic features and seizures (Sharp *et al.*, 2008). It then emerged that this same microdeletion occurs in 0.2% of patients with schizophrenia compared with 0.01% of controls (Stefansson *et al.*, 2008; Stone *et al.*, 2008). Finally, and with even higher frequency, the 15q13.3 microdeletion is present in 1% of patients with idiopathic generalized epilepsy (Helbig *et al.*, 2009). It is observed in a range of idiopathic generalized epilepsy subsyndromes including childhood absence epilepsy, juvenile myoclonic epilepsy, juvenile absence epilepsy and generalized tonic-clonic seizures alone. Family studies reveal that the 15q13.3 microdeletion behaves as would be expected for a susceptibility variant; it is found in some but not all family members with idiopathic generalized epilepsy and some unaffected members also carry the copy number variant (Dibbens *et al.*, 2009).

In the present issue, the European group responsible for identifying the role of the 15q13.3 microdeletion in idiopathic generalized epilepsy has expanded their studies to investigate the role of other recurrent copy number variants in this context (de Kovel *et al.*, 2009). In view of the previous association between 15q13.3 and many types of neuropsychiatric disorders, they examined five additional large recurrent microdeletions found in controls but occurring more frequently in schizophrenia, psychotic disorder, autism and mental retardation. Their association study of 1234 individuals with idiopathic generalized epilepsy compared with 3022 German controls yields positive results for the microdeletions at 15q11.2 (12 cases versus 6 controls) and 16p13.11 (6 cases versus 2 controls); and confirms their previous strong association with 15q13.3 and both inherited and *de novo* copy number variants. Overall, 8% (22/1234) patients have one of the five new microdeletions compared with 0.3% (9/3022) controls. Thus, the 15q13.3 microdeletion remains the most frequent of these hotspots identified to date in idiopathic generalized epilepsy ($\sim 1\%$ of cases), while the other microdeletions are rarer.

How do these microdeletions confer their pathogenic effect? This remains to be resolved but haplo-insufficiency of specific genes within a deletion is the favoured hypothesis. For example, the most promising suspect in the 15q13.3 microdeletion is *CHRNA7* which encodes the alpha 7 subunit of the nicotinic receptor. Other alternative mechanisms of pathogenicity include unmasking of recessive mutations in the remaining allele, epigenetic effects or sensitization to environmental influences. Given the phenotypic complexity associated with a single copy number variant, it is highly likely that many of these mechanisms are operating along with the genetic background of the individual.

Whilst testing for a specific microdeletion in idiopathic generalized epilepsy is not yet of clinical utility, further research

endeavours will further our understanding of the number and type of susceptibility variants that lead to a specific phenotype whether these be sequence changes or copy number variants. In the future, it is likely that a specific combination of susceptibility alleles will enable us to predict prognosis and therapeutic efficacy.

The new array single nucleotide polymorphism and comparative genomic hybridization microarray techniques shift the goal posts in that molecular geneticists are proposing a 'genotype first' methodology that overturns the traditional clinical approach of 'phenotype first'. The large scale of the molecular approaches in screening huge cohorts is exciting but the need for excellent phenotyping remains all the more important in order to bring meaningful interpretation to the emerging molecular discoveries. Ongoing collaboration between molecular scientists and clinicians will therefore be critical in solving the genetics of the idiopathic generalized epilepsies and improving outcomes for patients.

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