Molecular Genetics of Epilepsy

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by

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SUMMARY

To begin to understand the molecular basis of epilepsy it is necessary to identify the genes involved. One way of achieving this is to first localise a gene to a particular chromosome by genetic linkage analysis, then identify the actual gene involved using positional cloning or the positional candidate approach.

The first part of the project involved localising a gene that causes febrile convulsions (FEB). After testing 275 genetic markers throughout the human genome for linkage to the FEB gene, a locus on chromosome 8q13-q21 was found, where the multipoint lod score was 3.5 at D8S543. The gene was localised to a 10cM region between D8S1797 and D8S279. This was the first gene for febrile convulsions to be mapped. Five candidate genes in this region were excluded from causing febrile convulsions in this family. Two novel microsatellites were characterised to achieve this. The regional localisation of the gene is still too large to consider positional cloning.

An attempt was then made to localise the gene for adult-onset temporal lobe epilepsy (TLE). Two families were available for linkage analysis. Each family alone does not contain sufficient potentially informative meioses to justify a genome scan and combining the families for this purpose is risky due to the possibility of genetic heterogeneity. Therefore linkage analysis was limited to known candidate regions. Epilepsy genes have been mapped in the El mouse, a model for partial seizures like TLE, however the homologous regions in the human genome on chromosomes 2q, 3q, 6p, 7p, 11p, 15q and 20q were excluded. The places to which other human epilepsy genes map (6p, 8p, 8q, 10q, 19p, 20q and 21q were also excluded from causing TLE in these two families and the genetic basis of this disorder remains unknown.

The next part of the project involved attempting to identify epilepsy genes that had already been mapped. The gene for autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) maps to chromosome 20q13.2. Three candidate genes were present at this location, two neurotransmitter receptors (nicotinic acetylcholine receptor α4 subunit and neurotensin receptor) and one CNS specific hormone receptor (melanocortin-3 receptor). In collaboration with O. Steinlein in Germany, a mutation was found in the acetylcholine receptor, which alters a single amino acid in the ion channel of this cell surface protein. It was concluded that this mutation was the cause of ADNFLE in the family studied and thus the first idiopathic epilepsy gene identified.
Several types of acetylcholine receptors exist which are formed by varying combinations of α and β subunits of the protein. Therefore other acetylcholine receptor subunits were investigated for mutations in various different types of epilepsy. A silent (G to A) mutation in the β2 subunit was identified which is present in a larger proportion of epilepsy patients than in the general population. This may be an epilepsy susceptibility locus, however larger numbers of patients need to be screened to confirm this.

The gene in a family with benign familial neonatal convulsions (BFNC) maps to the same chromosomal band as ADNFLE on chromosome 20. The acetylcholine receptor α4 subunit had previously been excluded from causing BFNC in this family. A novel single nucleotide polymorphism was identified in the melanocortin-3 receptor. Recombination between this marker and BFNC excluded the melanocortin-3 receptor as the cause of this form of epilepsy. An allele of a minisatellite discovered in intron 2 of the neurotensin receptor was found to cosegregate with BFNC and this gene could not be excluded. However no mutations in the gene were identified using single strand conformation analysis.

The final part of the project involved localising the gene for generalised epilepsy with febrile seizures (GEFS). Using manual genotyping the gene was localised to chromosome 19q13.1 with a multipoint lod score of 3.85 at D19S414. The remainder of the genome was excluded by automated genotyping. A voltage gated sodium channel gene (SCN1B) found in this region was investigated as a possible candidate for GEFS and a mutation was promptly found. A G to C nucleotide substitution which changes a cystein to a tryptophan in exon 3 of the β subunit of this ion channel gene is believed to be the cause of GEFS in the family studied. This is the first gene to be associated with febrile seizures.

This project has involved the identification of two genes that cause idiopathic epilepsy. The ADNFLE gene is an acetylcholine-gated calcium channel and the GEFS gene is a voltage-gated sodium channel. Both of these genes code for proteins that form channels in the cell surface that control the flow of ions through the cell membrane. This is the basis for transmitting electrical impulses between nerve cells and the subtle mutations identified are presumed to be sufficient to cause the transient disruption of neural synchrony which ultimately leads to an epileptic attack.