Network: Systematic Gene Identification and Functional Analyses in Common CNS Disorders

Project: Gene Identification in Idiopathic Seizure Disorders

Armin Heils - Friedrich Wilhelms University, Dept. Human Genetics, Bonn - armin.heils@ukb.uni-bonn.de

Introduction

Epilepsy comprises a heterogeneous group of different seizure disorders which affect about 2% of the general population. Common epilepsy subtypes are idiopathic generalised epilepsy (IGE) and temporal lobe epilepsy (TLE). The most frequent form of seizure disorders are febrile convulsions (FC) although not regarded as an epilepsy syndrome in a narrow sense. (Commission on Classification and Terminology of the International League Against Epilepsy) .
Common forms of IGE including childhood and juvenile absence epilepsy (CAE, JAE), juvenile myoclonic epilepsy (JME), and epilepsy with grand mal seizures on awakening (EGMA) affect about 0.6% of the population world-wide and are thus a major challenge for current biomedical research concepts. While several disease-causing mutations have already been identified in rare monogenic forms of IGE, only little is known about genes and the molecular mechanisms playing a role in the etiology and pathophysiology of common IGE syndromes.

The same applies for FC. Despite the discovery of several FC disease loci identified in few large multigenerational families, the molecular genetic basis of FC remains elusive so far. However, the significance of identifying genes causing common forms of FC is reflected by the fact that up to 30% of all TLE patients report a history of FC during childhood. The elucidation of the close relationship between FC and TLE may thus open novel research directions towards the identification of genes contributing to the etiology of TLE which accounts for about 30% of all epilepsies.

TLE has traditionally been regarded as an exogenous disease with genetic factors playing only a limited causative role. However, this point of view has recently been questioned by the identification of *LGI1* mutations causing non-lesional lateral TLE (Kalachikov et al., 2002) Therefore, it is likely that so far unknown genes may contribute to increased seizure liability in the majority of common epileptic disorders even beyond clinically delineated syndromes.

As linkage studies and positional cloning strategies have been so tremendously successful in rare monogenic epilepsies, a similar but modified approach has recently been applied in common forms of IGE. Results of a systematic search for IGE susceptibility genes (Sander et al., 2000) provided evidence for the existence of three chromosomal IGE susceptibility loci allowing the identification of a first gene playing a role in the etiology of all common IGE subtypes (please see: results). These findings provided further evidence that linkage studies in genetically complex disorders may finally lead to the identification of disease causing genes.

A second complementary approach is based on physiological considerations rather than positional evidence, but most of the candidate gene studies assessing genes solely selected on the basis of their physiological role did not provide a major step towards the identification of disease-associated sequence variation conferring susceptibility to IGE. Nevertheless, results of a recent study may have identified major neuronal pathways involved in epileptogenesis. In this study mutations in *CACNA1H* encoding a T-type Ca^{2+} channel have recently been identified in patients with CAE (Chen et al., 2003). This ion channel as well as hyperpolarization-activated cation channels, *HCN1-4*, play an important role in the generation of rhythmic thalamo-neuronal activity which has been implicated in the etiology of CAE. Together with three known T-type Ca^{2+} channels encoded by *CACNA1G, CACNA1H,* and *CACNA1I* these genes may well be regarded as attractive candidate genes for CAE.
Several ion channel mutations have already been identified in rare monogenic seizure disorders. Up to now none of these genes including those encoding neuronal Na^{+} and K^{+} channels as well as nicotinic acetylcholine receptors (nACHR) have been shown to be involved in common forms of epilepsy. Only recently, a mutation in the alpha1 subunit of gamma-amino-butyric-acid (GABA)A receptors has been identified in a family with JME (Cossette et al. 2002). By screening our patient samples we detected a second *de novo GABRA1* mutation in a single patient with CAE (please see: own previous work). These findings together with other results from our own laboratory support the neurobiological concept that genetically driven impairment of synaptic inhibition may play a key role in the etiology of idiopathic seizure disorders.

Results

The realization of our studies is primarily based on the availability of large patient samples, genome-wide linkage studies and fine-mapping strategies, a systematic search for mutations and polymorphisms as well as on functional studies. Together with T. Sander, Berlin, we have collected a large number of patients and families with different IGE syndromes. Together with other collaborating groups we have increased the number up to 750 IGE patients/families. A large study sample comprising 280 TLE patients is also available as well as a study sample comprising 255 FC patients/families.

These large family samples are an important prerequisite for successful disease gene identification. This has recently been demonstrated by collaborative efforts between our group and those of T. Sander and H. Lerche. A first step towards successful identification of genes involved in IGE has been achieved by the results of a systematic genome search including 130 IGE multiplex families (Sander et al., 2000) which provided evidence for the existence of at least three chromosomal IGE susceptibility loci. Significant linkage evidence was obtained for chromosome 3q26. Funded by NGFN we were able to identify the chromosome 3q26 *CLCN2* encoding the voltage-gated chloride channel ClC-2 as the first as yet described disease gene, sequence variants of which can contribute to the etiology of all four common IGE syndromes (Haug et al., 2003). Mutations identified were functionally characterized by H. Lerche and his co-workers who could show that ClC-2 mutations may lead to neuronal hyperexcitability via an impairment of gaba-ergic synaptic inhibition.

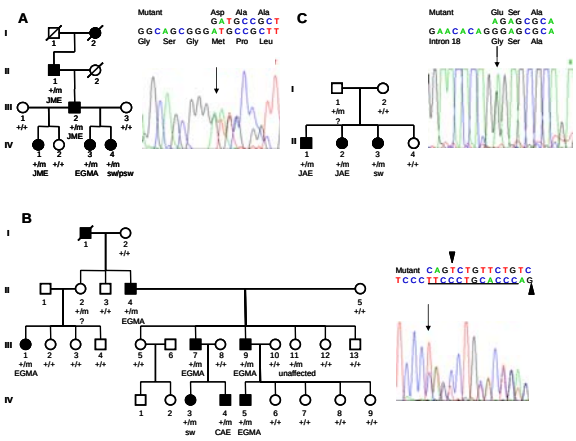

Fig 1: *IGE Families identified with CLCN2 mutations.*

Outlook

In a first step towards the identification of further IGE and FC susceptibility genes, we will continue narrowing down three chromosomal regions of positive linkage findings in IGE (2q36, 3q26 and 14q23, Sander et al., 2000) as well as those expected through currently conducted additional genome-wide linkage studies in FC and IGE (Heils, Sander). Since chromosomal regions of interest may span about 30 cM, we aim to narrow down the disease loci by further fine-mapping strategies as well as by a systematic SNP-based linkage disequilibrium mapping approach assessing large case-control samples. More than 1000 healthy controls (genomic controls) are available for this approach *(Genome scans and fine - mapping)*.

As soon as we will have identified putative disease genes we will systematically search for disease-causing sequence variants by a large scale sequencing approach. We will determine the population frequency in a sufficient number of control chromosomes, and in addition, a disease-causing role of a given sequence variant will also be tested by genotyping further family members and the degree of co-segregation between the affection status of respective family members and the sequence variant under investigation. However, these strategies have been proven to be successful especially in monogenic disorders. We will therefore also conduct population-based and family-based association studies in independent samples to clarify the relative risk contribution of different mutations and/or polymorphisms by considering the haplotype architecture in respective genes and / or chromosomal regions *(Positional candidate gene studies)*.

As outlined above we also aim to conduct functional candidate gene studies emerging from recent findings in IGE. We will assess the role of different calcium channel genes as well as of genes involved in inhibitory gaba-ergic pathways. The methods applied will be the same as those applied in our positional candidate gene studies *(Functional candidate gene studies)*. The analysis of linkage data and candidate gene study results requires biometrical and bioinformatic expertise and support. This will be provided in part by T. Sander but mainly by the GEM Bonn.

The identification of putative disease-causing genes will be followed by (i) testing the prevalence of a given mutation / polymorphism in different seizure disorders and (ii) functional studies. *(Functional studies)*.

Lit.: ***1.*** *Kalachikov S etal. Mutations in LGI1 cause autosomal dominant epilepsy with auditory features. Nat Genet. 2002 Jan 28;30:335-41.* ***2.*** *Sander T et al. Genome search for loci of common idiopathic generalised epilepsies. Hum Mol Genet. 2000 Jun 12;9:1465-72.* ***3.*** *Cossette P. et al. Mutations of GABRA1 in an autosomal dominant form of juvenile myoclonic epilepsy. Nat Genet. 2002 May 6;31:184-9.* ***4****. Haug K et al. Mutations in CLCN2 encoding a voltage-gated chloride channel are associated with idiopathic generalized epilepsies. Nat Genet. 2003 Mar3;33:527-32.*