

**Network: Systematic Gene Identification and Functional Analyses in Common CNS Disorders****Project: Molecular Genetic Dissection of Idiopathic Generalised Epilepsy**

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**Introduction**

Idiopathic generalised epilepsies (IGEs) affect about 0.6% of the general population and account for 30% of all epilepsies [1]. The clinical feature of IGE syndromes is characterised by the age-related occurrence of recurrent unprovoked generalised seizures in the absence of detectable brain lesions or metabolic abnormalities [2]. Childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy (JME) and epilepsy with generalised tonic-clonic seizures (EGTCS) are the most common IGE subtypes [2]. The leading seizure types of these IGE syndromes are absence seizures (CAE, JAE) and bilateral myoclonic seizures on awakening (JME). The electroencephalographic (EEG) signature of IGE seizures is characterised by generalised spike-wave discharges (GSW-EEG), which reflect a synchronised hyperexcitability state of thalamo-cortico-thalamic circuits [3].

The aetiology of common IGE syndromes is genetically determined and likely represents a biological continuum, in which a small fraction follows monogenic inheritance, whereas the majority of common IGE syndromes display an oligogenic or polygenic predisposition [4]. Twin and family studies indicate an overlapping genetic component that is shared across common IGE syndromes, but also provide evidence that distinct gene configurations determine the phenotypic expression of certain seizure types, such as absence and myoclonic seizures [5,6]. Up to date, 19 genes have been identified in predominantly monogenic forms of idiopathic epilepsy [7]. The majority of epilepsy genes identified so far encode voltage-gated or ligand-gated ion channels ("channelopathies"; e.g. *CHRNA4/B2*, *KCNQ2/3*, *SCN1A/2A/1B*, *GABRA1/G2*, *GABRD*, *CLCN2*, *CACNA1A*, *CACNB4*), whereas others (*EFHC1*, *LG11*, *ME2*, *BRD2*) seem to impair critical control points that balance the developmental assembly of inhibitory and excitatory circuits. Although the known epilepsy genes display considerable genetic heterogeneity and explain only a small proportion of the genetic variance, the identified mutations trace functionally convergent pathways of epileptogenesis and define pharmacogenetic targets. However, none of the gene mutations identified to date confers a frequent and substantial effect to the genetic variance of common IGE syndromes.

Recent linkage mapping of loci predisposing to common IGE syndromes revealed several tentative susceptibility loci in the chromosomal regions 2q36, 3q26, 5p15, 5q22, 6p12, 6p21.3, 8p12, 8q24, 13q31, 14q23, 15q14 and 18q21. These findings provided important positional clues for the identification of the first genes (*CLCN2*, *EFHC1*, *BRD2*, *ME2*) involved in the pathogenesis of common IGE syndromes [7]. For most implicated regions, replication studies failed to establish unequivocal linkage relationships due to the confounding influences of phenotypic variability, complex inheritance and genetic heterogeneity [8]. An attempt to reduce complexity and genetic heterogeneity is the usage of neurobiologically defined traits, such as certain seizure types or photosensitivity, instead of syndrome categories. These "endophenotypes" are suitable to dissect the phenotypically complex IGE syndromes and to specify phenotype-genotype-relationships that are closer to the effect of the underlying susceptibility gene.

The present genome-wide linkage scans aim to dissect IGE traits that differentially confer susceptibility to either absence or myoclonic seizures and photosensitive IGE.

**Results**

The study sample included 93 European IGE-multiplex families with 469 family members, of which 416 individual members were genotyped. The families were ascertained through a proband with either CAE, JAE or JME, and one or more siblings affected by an IGE trait. The study protocol was approved by the institutional review boards of the participating European centers. Written informed consent was obtained from all participants. Clinical and EEG data for each participant was documented in standardised anonymous protocols. The diagnoses of IGE syndromes were performed according to the current classification of the International League Against Epilepsy [2]. The EEG assessment of photoparoxysmal response (PPR) on intermittent photic stimulation (IPS) applied internationally recommended guidelines as described previously [9]. The IPS procedure offers a controllable experimental setting to determine the individual liability to abnormal cortical synchronisation and to investigate mechanisms that are involved in the transition to an epileptic state [10]. To dissect seizure type- and PPR-related susceptibility genes, we defined three distinct subgroups of families distinguished by their predominant seizure type or an abnormal PPR.

The genome scan included 630 microsatellite polymorphisms covering the entire autosomal genome and the X-chromosome with an average spacing of 5.3 cM. Multipoint nonparametric linkage (NPL) analyses were performed using the ALLEGRO program [11]. To determine significance levels of the observed NPL results, simulation analyses were carried out under the null hypothesis of no linkage to calculate empirically genome-wide type-I error rates. NPL scores occurring once by chance in a genome-wide scan were considered as suggestive evidence for linkage. Significant linkage refers to a NPL score that occurs 0.05 times randomly.

The genome-wide NPL scan for IGE susceptibility loci in the entire sample of 93 IGE-multiplex families yielded a complex pattern of NPL signals in the chromosomal regions 5q31, 11q13, 13q31 and 19q13. The novel IGE locus on chromosome 13q31 reached empirical genome-wide evidence for significant linkage, whereas the other NPL peaks in the chromosomal regions 5q31, 11q13 and 19q13 exceeded significance thresholds of suggestive evidence for linkage. Concordant parametric linkage results strengthened the validity of the NPL findings. Parametric LOD score analyses revealed significant linkage evidence in the chromosomal region 19q13, and evidence for suggestive linkage on chromosomes 11q13, 13q31, 14q11 and 19p13.

To search for seizure type-related susceptibility loci, a narrow trait definition that considered only family members with the core seizure type as "affected" was tested in the corresponding family subgroup. To search for susceptibility loci related to typical absence seizures, we selected 40 IAE-multiplex families without JME members, containing at least two siblings with either childhood (CAE) or juvenile (JAE) absence epilepsy. When family members with typical absence seizures were classified as "affected", parametric and nonparametric linkage results provided consistent evidence for two loci predisposing to typical absence seizures in the chromosomal regions 7p14 and 13q31. Interestingly, a quantitative trait locus for generalised spike-and-wave discharges in the GAERS (genetic absence rats from Strasbourg) rat model of idiopathic absence epilepsy (*swd1/gaers1*) was mapped by homology to the human chromosomal region 7p14-p15 [12], where we found linkage

to absence seizures. The epilepsy phenotype in GAERS rats is genetically determined and resembles many of the characteristics of human absence epilepsy. These findings support evidence that the region 7p14 harbors a susceptibility gene involved in the generation of synchronised oscillations in thalamocortical networks.

To scan for genes predisposing preferentially to myoclonic seizures, we selected 21 families of JME patients, in which at least one other sibling was affected by either JME or epilepsy with generalised tonic-clonic seizures on awakening. When family members with either myoclonic or generalised tonic-clonic seizures on awakening were considered as "affected", linkage analyses provided evidence for two susceptibility loci on chromosomes 6p12 and 19q13 in the JME families. The candidate region 6p12 harbours the *EFHC1* gene that was identified as susceptibility gene for JME [13].

To dissect loci that confer susceptibility to photosensitive IGE, we selected 25 families, in which PPR was strongly associated with IGE. MOD-score analyses provided significant evidence for linkage to the region 13q31, when the trait definition classified family members with either PPR or IGE as "affected" [14]. Taking into account that 84% of the family members with IGE also exhibited PPR, and PPR might have been missed in some of the IGE subjects due to its age-dependent manifestation and suppression of PPR by antiepileptic medication, this strong phenotypic association supports the conclusion that the locus on 13q31 contributes a major gene effect to an epileptogenic pathway that is shared by both PPR and IGE.

#### *In conclusion:*

- Our results display a complex pattern of linkage signals on 5q31, 11q13, 13q31 and 19q13 in the entire sample of IGE-multiplex families.
- The pattern of linkage hints differs depending on the predominant seizure type in the families.
- Susceptibility loci on 7p14 and 13q31 seem to confer susceptibility to absence seizures, whereas loci on 6p12 and 19q13 preferentially predispose to myoclonic seizures on awakening.
- PPR represents a suitable endophenotype to disentangle the complex and heterogeneous pathways of epileptogenesis [10,14]. The novel susceptibility locus on chromosome 13q31 is likely to contribute a major gene effect to an epileptogenic pathway that is shared by both PPR and IGE.

#### Outlook

Our present genome scans demonstrate that the specification of neurobiologically defined IGE traits facilitate the molecular genetic dissection of common IGE syndromes. The inconsistent replication of linkage claims implies that the genetic architecture of common IGE syndromes is more complex and heterogeneous than optimistically expected. Three clues will be essential for dissecting the oligogenic predisposition of common IGE syndromes. First, clinical genetics need to delineate suitable endophenotypes and to specify phenotype-genotype-relationships that are closer to the effect of the underlying susceptibility gene. Second, linkage statistics have to take into account gene interactions. Third, large multicenter collaborations using standardised protocols for phenotyping of IGE traits are imperative to achieve sufficient power to disentangle the complex genetic basis of IGE traits. Even if genetic diversity will hinder the dissection of the oligogenic predisposition of common IGE syndromes, we are confident that most molecular defects will functionally converge on a few common processes regulating cortical synchronisation [15]. Characterisation of core molecular pathways would advance our understanding of epileptogenesis, and may have equally significant therapeutic implications.

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