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# Evidence of linkage to chromosome 5p13.2-q11.1 in a large inbred family with Genetic Generalized Epilepsy

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**Summary**

The clinical genetics of Genetic Generalized Epilepsy (GGE) suggest complex inheritance; large pedigrees, with multiple affected individuals, are rare exceptions. We studied a large consanguineous family from Turkey where extensive electro-clinical phenotyping revealed a familial phenotype most closely resembling Juvenile Myoclonic Epilepsy. To be considered affected (n=14), a diagnostic EEG was required. Seizure onset ranged between 6-19 years (mean=12 years). 13/14 experienced myoclonic jerks; in 11, this was associated with eyelid blinking and in 10, interspersed with absences. Generalized tonic-clonic seizures (GTCS) were seen in 11. One individual had GTCS alone. EEGs demonstrated generalized polyspike and wave discharges that were not associated with photoparoxysmal response. Intellect was normal. 19 family members were subsequently chosen for nonparametric multipoint linkage analyses, which identified a 39.5Mb region on chromosome 5 ($p<0.0001$). Iterative analysis, including discovery of a subtly affected individual, narrowed the critical region to 15.4Mb and possibly to 5.5Mb. Homozygous versus heterozygous state of the refined 5p13.2-q11.1 haplotype was not associated with phenotypic severity or onset age suggesting that one versus two pathogenic variants may result in similar phenotypes. Whole-exome sequencing (n=3) failed to detect any rare, protein-coding variants within the highly significant linkage region that includes *HCN1* as a promising candidate.

**Introduction**

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The Genetic Generalized Epilepsies (GGEs) are one of the commonest forms of epilepsy. Evidence for their genetic etiology is a recurrence risk ratio of 4-9[1] compared to the general population and very high concordance in monozygotic twins.[1] These data, together with pedigree analyses, suggest most cases are due to complex inheritance, involving two or more genes. Consistent with this, recurrent copy number variants (CNVs) at 15q13.3, 15q11.2 and 16p13.11 are established as important GGE risk factors and earlier studies have associated variants in *EFHC1*, *CACNB4*, *GABRD, CLCN2*, *BRD2*, *GJD2*, *NEDD4L* and *ME2* with Juvenile Myoclonic Epilepsy (JME) more specifically (see Supplementary references 4,5,9-16). However, replicating these findings and identifying new risk alleles has proven challenging with more recent large-scale exome sequencing efforts[2] and genome-wide association studies[3] failing to confirm these claims.

Although less common, Mendelian forms of GGE are recognized with dominant *SLC2A1*[4] and *GABRA1*[5] mutations reported in rare families. Family studies, where feasible, have proven powerful in the identification of Mendelian epilepsy genes. In particular, linkage studies have been of great value in localizing genes with rare mutations of large effect leading to monogenic disorders.[1] The advent of next generation sequencing, initially via whole-exome sequencing (WES), and now, more increasingly by whole-genome sequencing (WGS), has led to the expeditious discovery of many of the underlying causal variants in linkage regions.[6] As such, large kinships with multiple affected family members and a Mendelian inheritance pattern may facilitate gene discovery in rare GGEs.

To further understand the genetic architecture of GGE, we studied a large family from south-eastern Turkey with a pedigree suggestive of Mendelian inheritance and report the linkage analysis.

## Methods

### *Patients*

The proband and his available family members underwent a detailed clinical interview by phone and in person. The study involved neurological examination and a

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3 hour 21-channel EEG recording, which included hyperventilation and intermittent photic stimulation. Medical records were also collected.

Diagnoses were made in accordance with the International League Against Epilepsy classification and terminology;[7, 8] for an individual to be considered affected, a diagnostic EEG was required.

The study was approved by Istanbul University's institutional review board and by the Human Research Ethics Committee, Austin Health. All participating patients and family members gave informed consent.

*Molecular genetic analysis*

DNA was obtained from as many family members as possible (Supplementary methods). 19 family members (14 affected and 5 unaffected) were chosen for single nucleotide polymorphism (SNP) genotyping using the Illumina Infinium HumanOmniExpress BeadChip microarray (Australian Genome Research Facility, Melbourne, VIC, Australia).

A subset of 7,353 highly informative SNPs were chosen for linkage mapping across the 22 autosomal chromosomes (Supplementary methods). To account for the exceptionally large pedigree we employed an approximate nonparametric linkage analysis with MORGAN using the lm_ibdtests option. Three analyses, with different starting points, were run for each chromosome consisting of 10,000 burn-in and 100,000 Monte Carlo iterations.[9] Final p-values for the $S_{robdom}$ test statistic were averaged across the three runs.

The complete set of genotyped SNPs on chromosome 5 were analysed to verify the linkage breakpoint coordinates. This also enabled the SNP data for the two putative phenocopies to be interrogated for smaller identity-by-descent (IBD) or homozygosity-by-descent (HBD) regions of the shared chromosomal segment that may have gone undetected by the subset of SNPs used in the linkage analysis.

CNV and WES analyses were subsequently performed (Supplementary methods).

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**Results**

*Clinical presentation*

Fourteen family members with seizures and generalized epileptiform discharges on EEG (6 male : 8 female) were initially evaluated. The mean age of seizure onset was 12 years (range 6-19 years) with all affected family members reporting fairly homogenous GGE characteristics (Table 1). The familial phenotype most closely resembled JME. Neurological examination and magnetic resonance imaging of the brain were normal in all.

The family was identified through the proband (Figure 1, VII-10) who had seizure onset at 12 years with frequent episodes of eyelid blinking and brief absences, sometimes associated with myoclonic limb jerking, and in some instances evolving into GTCSs on awakening or soon after falling asleep. Seizures were precipitated by sleep deprivation, fatigue and medication non-compliance. He had no GTCSs from 17 years to 22 years of age on valproate and levetiracetam, but showed rare eyelid blinking episodes with sleep deprivation. He had two additional GTCSs associated with medication noncompliance when he was 23 years old. His EEG showed generalized polyspike-and-wave discharges that were not associated with photoparoxysmal responses or eye-closure abnormalities (Supplementary Figure 1); an absence seizure was recorded during hyperventilation at 16 years.

Overall, affected family members presented with a similar electro-clinical history and the prognosis was favourable (Supplementary results). Four of 14 individuals had an earlier onset of seizures, but were otherwise indistinguishable from other family members (Table 1). Of note, individual VII-15 had seizure onset at 19 years with GTCS on awakening; isolated absences and myoclonus were denied. She also had the diagnosis of facioscapulohumeral muscular dystrophy.

Two additional family members (VI-8, VII-9) were clinically affected, but insufficient information was available (Supplementary results); they were excluded from genetic analyses.

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*Genetic analyses*

Non-parametric linkage analysis revealed a single region of approximately 39.5 Mb at chromosome 5p13.2-q13.3 (p-value <0.0001; Supplementary Figure 2) where 12 affected family members, and a single unaffected family member, shared one or two copies of the putative disease haplotype (a) (Figure 1). Two affected individuals (VII-1 and VII-15) did not inherit this 5p13.2-q13.3 linked region (haplotype a) and were considered likely phenocopies.

To determine the exact start and end coordinates for the 5p13.2-q13.3 haplotype we manually examined the complete set of genotyped SNPs shared by affected family members, including the two putative phenocopies, across the region. This process revealed two smaller IBD segments of the same ancestral haplotype within the pedigree (5p13.2-q11.1 and 5p12-q11.1) (Supplementary Figure 3); one of which was present in VII-1. The 5p13.2-q11.1 haplotype (b) was inherited by VII-1 from her father who was reported to be unaffected (VI-2) (Figure 1). Further questioning of VI-2 revealed a history of rare myoclonus from age 16 years, and a subsequent EEG demonstrated brief generalized spike and waves, confidently refining the region of interest to 5p13.2-q11.1.

The 5p13.2-q11.1 locus encompasses 15.4Mb of the genomic region and harbors 114 genes including *HCN1*, which has been previously associated with epilepsy.[10] The alternate 5p12-q11.1 haplotype (c) of 5.5Mb encompasses just three genes, again including *HCN1* (Supplementary Figure 3). We cannot, however, confidently limit the region of interest to this segment as no affected individual carries this smallest version of the putative disease haplotype alone (Figure 1). Heterozygous carrier VI-10, had a normal 3hr EEG examination and was clinically unaffected on close questioning.

Penetrance for the refined 5p13.2-q11.1 region (Figure 1; haplotypes a and b) is 93% with one unaffected homozygous carrier (VI-5) (Figure 1). The homozygous versus heterozygous state for the haplotype was not associated with phenotypic severity or age of seizure onset (Table 1). Affected family member (VII-15) remains unlinked to the chromosome 5 region.

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No plausible disease-causing variants were identified (Supplementary results).

**Discussion**

The present study describes the electro-clinical features of a large consanguineous family from south-eastern Turkey with GGE and significant linkage to chromosome 5p13.2-q11.1. Importantly, we define three versions of the same chromosome 5 haplotype that have derived from a common ancestor, been refined by different recombination events through the generations, and brought together in this nuclear family through complex inbreeding (Figure 1 and Supplementary Figure 3). The seizure semiology reported by affected family members was most consistent with JME. As GGE usually follows a complex pattern of inheritance most families with JME do not show Mendelian transmission,[11] however rare autosomal dominant families have been reported,[5] and the possibility of recessive families has been raised.[12]

Although JME is characterized by adolescent onset, myoclonic jerks on awakening, accompanied by GTCSs in 80-95% of patients, and in a third of patients by typical absence seizures, its diagnostic boundaries are not rigidly defined.[13] The phenotype in this family was fairly homogeneous, however, age of onset was relatively broad with four individuals having early onset. Whilst over 75% of patients with JME have their first seizures between the ages of 12 and 18 with a mean age at onset around 14,[11] this observation is consistent with previous reports of early onset JME syndrome before age 10.[14] The four individuals with early onset of seizures had no photoparoxysmal response or eye-closure abnormalities in their EEGs, nor was it associated with a particular clinical presentation or prognosis.

Linkage analysis in a common condition such as epilepsy can be challenging due to incomplete penetrance and phenocopies. Presence of the putative disease haplotype in individual VI-5, who is still asymptomatic at age 50 years, suggests that the penetrance is high but incomplete in this family. There was also one affected individual (VII-15) who did not link to the chromosome 5 region. The clinical phenotype for VII-15 was somewhat distinguishable from other family members, with late seizure onset at 19 years with GTCS alone (Table 1).

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All other affected family members were shown to share either one or two copies of the refined 5p13.2-q11.1 haplotype likely reflecting that inbreeding can enrich for disease alleles from common ancestors leading to a higher penetrance than expected for a dominant disorder. Our results also indicate that mutations in the heterozygous versus homozygous state may result in similar phenotypes. Whilst this has been reported before, for example in Huntington's disease,[15] it is a novel concept for epilepsy genetics.

Alternatively, it may be that this interesting observation is consistent with complex inheritance and the chromosome 5 haplotype is harbouring a risk as opposed to a dominant allele. Whilst the large number of affected family members is suggestive of a Mendelian disorder, the multiple consanguineous unions (leading to a less diverse genetic background) may instead have been ripe for a complex genetic disorder to mimic as Mendelian. The phenocopy (VII-15) may therefore still be sharing some risk alleles with the nuclear family, but not all (i.e., the chromosome 5 allele).

One promising gene located within the chromosome 5 haplotype has been previously associated with epilepsy. Heterozygous mutations in *HCN1* can cause early infantile epileptic encephalopathy.[10] This gene quickly became our top candidate, particularly as an excess in ultra-rare variation in genes known to cause severe epilepsies has been recently associated with familial GGE.[2] Whilst we have confidently excluded protein-coding mutations in *HCN1*, future investigations should focus on non-coding regions.

The genetic basis to the GGEs remains elusive. Whilst next generation sequencing has revolutionized genetic research particularly in monogenic disorders, progress in common complex diseases has been slower. Here, having excluded protein-coding variants in genes and CNVs previously associated with GGE, we argue that large pedigrees remain valuable in the quest for novel gene discovery. With the search space narrowed to a single region of high significance on chromosome 5 we can target future efforts with confidence.

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**Figure legends**

Figure 1.

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Family pedigree detailing complex inbreeding loops and segregation of the three versions of the chromosome 5 ancestral haplotype present in either IBD or HBD segments.

Supplementary Figure 1.
EEG of patient VII-10 showed normal background activity with generalized polyspike and wave discharges.

Supplementary Figure 2.
Approximate genome-wide $S_{robdom}$ values obtained by averaging the three runs of the MORGAN routine lm_ibdtests with different starting points following 100,000 iterations.

Supplementary Figure 3.
Summary of the three chromosome 5 shared ancestral haplotype segments a, b, c.

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**Disclosure of conflicts of interest**
None of the authors has any conflict of interest to disclose.

**Ethical publication statement**
We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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**Tables**

Table 1. Clinical summary of family members with generalized epileptiform activity on EEG that were included in the linkage analyses.

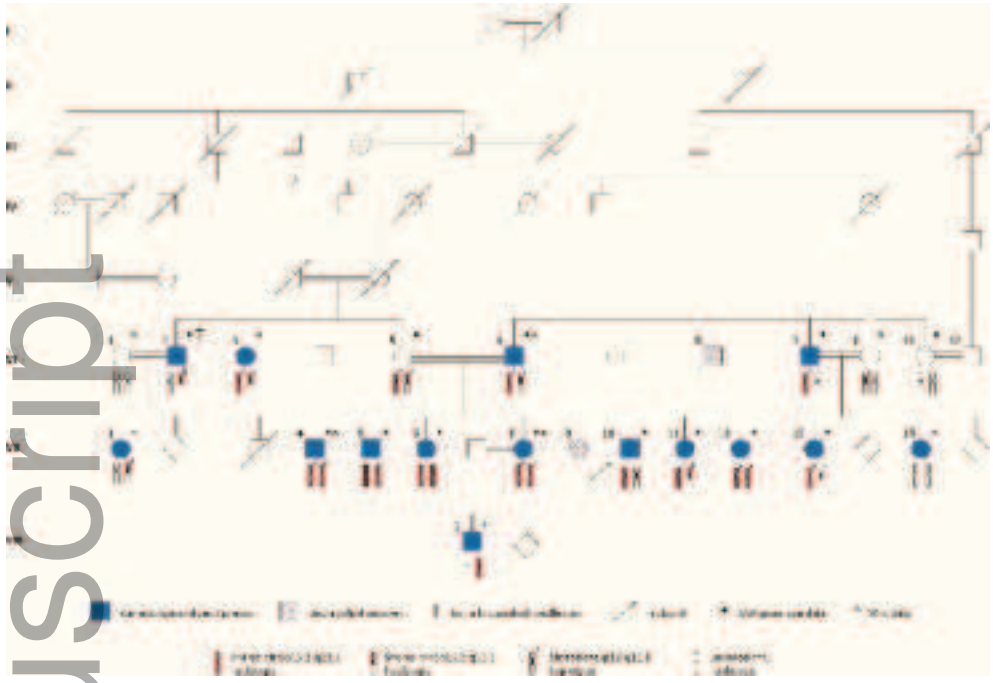
| | Onset age (yrs) | Myoclonic jerking | Eyelid blinking | Absence seizures | GTCS | Other | AEDs |
|---|---|---|---|---|---|---|---|
| VI-3 | 14 | + | + | + | + | - | VPA |
| VI-6 | 12 | + | + | + | - | - | VPA |
| VI-9 | 15 | + | + | + | + | - | - |
| VII-1 | 12 | + | + | - | + | - | VPA |
| VII-4 | 13 | + | + | + | + | - | VPA |
| VII-5 | 12 | + | - | + | + | - | VPA |
| VII-6 | 15 | + | - | + | + | - | VPA, LEV |
| VII-8 | 14 | + | + | + | + | - | VPA |
| VIII-1 | 6 | + | + | + | - | - | VPA |
| VII-10 | 12 | + | + | + | + | - | VPA, LEV |
| VII-11 | 7 | + | + | + | + | - | VPA |
| VII-12 | 7 | + | + | - | + | - | VPA |
| VII-13 | 8 | + | + | - | + | FS | VPA |
| VII-15 | 19 | - | - | - | + | FSHD | VPA |
| VI-2† | 16 | + | - | - | - | - | - |

† initially classified unaffected; - absence of clinical symptom; + presence of clinical symptom; FSHD- facioscapulohumeral muscular dystrophy; FS – febrile seizures; AEDs – anti-epileptic drugs at the time of study; VPA – valproate; LEV - levetiracetam

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