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Genetic linkage study of an autosomal recessive form of juvenile myoclonic epilepsy in a consanguineous Tunisian family

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Summary Juvenile myoclonic epilepsy (JME) is the most common idiopathic generalized epilepsies (IGEs), affecting 12—30% of all epilepsies in medical centers. To date genetic linkage studies have revealed putative loci on different chromosomes, but these findings are still inconclusive about which gene precisely is responsible for the disease. Here, we report the genetic and clinical analysis of a (JME) consanguineous Tunisian family with four affected children out of eight. A genome-wide search was carried out by using the Affymetrix GeneChip Mapping 500K NspI chip. Pairewise logarithm of the odds (LOD) scores were calculated with MERLIN (1.1) assuming an autosomal recessive model, and a complementary homozygous mapping analysis was performed with AutoSNPa software. The genome-wide parametric linkage analysis showed suggestive linkage to chromosome 2q. Interactive visual analysis of SNP data using AutoSNPa revealed two large regions of shared homozygosity by descent on 2q23.3 and on 2q24.1. We decided to sequence the exons of the two genes coding for such proteins located in 2q23.3, CACNB4 and 2q24.1, KCNJ3. No nucleotide variation — comprising the previously reported mutations — was detected.

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Introduction

Juvenile myoclonic epilepsy (JME) is the most common form of idiopathic generalized epilepsies (IGEs), affecting 12—30% of all epilepsies in medical centers (Janz and Christian, 1957; Panayiotopoulos et al., 1994; Delgado-Escueta and Enrile-Bacsal, 1984). It is characterized by myoclonia, generalized tonic—clonic seizures (GTCS), and, less frequently, absences. Its seizures occur predominantly at awakening and are associated with clear precipitating factors, such as sleep deprivation, fatigue, and alcohol intake. Its electroencephalography (EEG) findings show generalized 4—6 Hz irregular spike- or polyspike-wave activity, with a maximum in frontocentral regions (Janz and Christian, 1957; Martínez-Juárez et al., 2006).

JME can be inherited as a Mendelian autosomal dominant (Cossette et al., 2002) or autosomal recessive trait (Panayiotopoulos and Obeid, 1989) or as a non-Mendelian complex genetic trait (Gardiner, 2005). To date genetic linkage studies have revealed putative loci on chromosomes 6p21.3 (HLA region) (Greenberg et al., 1989; Weissbecker et al., 1991; Sander et al., 1997a; Pal et al., 2003; Lorenz et al., 2006), 6p12-11 (Liu et al., 1996; Serratosa et al., 1996; Bai et al., 2002), 15q14 (Elmslie et al., 1997; Taske et al., 2002), 10q25-26 (Puranam et al., 2005), 16p13 and 7q32 (Pinto et al., 2005), and 5q12-q14 (Kapoor et al., 2007). Three mutation-harboring Mendelian genes for JME have been reported. Mutations in α1 subunit of γ-aminobutyric acid receptor subtype A ($GABA_A$) on chromosome 5q34 segregated with nine affected individuals of a three-generation French Canadian family with JME (Cossette et al., 2002). A mutation in a chloride-channel gene, CLCN2, on chromosome 3q26 was reported in a German JME patient (Kleefuss-Lie et al., 2009). Heron et al. (2007) reported a variant in a subunit of the calcium channel gene (CACNA1H) in JME patients, showing a slight reduction in ion channel function. These results suggest that, as for other IGEs, genes coding for ion-channels are good candidates for JME. On the 6p12 JME locus (Liu et al., 1996; Serratosa et al., 1996), mutations were identified in a gene that encodes a protein with one EF-hand motif, hence called EFHC1 (Suzuki et al., 2004; Annesi et al., 2007; Medina et al., 2008). Finally, in the 6p21.3 region a linkage disequilibrium between JME and a core haplotype peaking in the BRD2 (RING3) gene has been reported in different populations (Pal et al., 2003), but these data are still inconclusive about which gene precisely and what phenotype (JME) or associated trait such as visual sensitivity (de Kovel et al., 2007).

Here, we report the genetic and clinical analysis of a JME consanguineous Tunisian family with four affected children out of eight.

Materials and methods

Patients and genomic DNA

The patients were four children born to healthy first-cousin parents of Tunisian origin (Fig. 1). All individuals were clinically assessed at the Department of Clinical Neurophysiology from Salhoul's Hospital (Sousse, Tunisia) and were considered as affected if they showed one or more myoclonic jerks. We collected information from affected patients about their history of seizures, age at onset, duration and type of seizures, number of seizures, intellectual outcome, antiepileptic drug therapy, and seizures outcome. Electroencephalographic (EEG) with photic stimulation was obtained for subjects (IV-1, IV-4, IV-5, and IV-6), respectively.

After informed consent was obtained from all family members of from their legal representatives, venous blood samples were collected, except for individuals (II-1, IV-3, IV-4 and IV-7) who were unavailable the day of collection. Genomic DNA was extracted from peripheral blood mononuclear cells using the standard salting out procedure (Miller et al., 1988). This study was approved by the Research and Ethics Review Board of the Department of Neurology, University Hospital of Geneva. The study procedures were designed and performed in accordance with the declaration of Helsinki.

Genetic linkage analyses

A genome-wide search was carried out by using the Affymetrix GeneChip Mapping 500K NspI chip®, which allowed to genotype around 260,000 SNPs (http://www.affymetrix.com). The arrays were scanned with the GeneChip Scanner 3000 7G® and the data were processed with GeneChip Operating Software (GCOS, ver. 1.4) and GeneChip Genotyping Analysis Software (GTYPE, ver. 4.1).

Pairewise logarithm of the odds (LOD) scores were calculated with MERLIN (1.1) assuming an autosomal recessive model.

A complementary homozygous mapping analysis was performed with AutoSNPa software (Carr et al., 2006).

Mutational analysis

Candidate genes were identified using the UCSC Genome Browser (http://genome.ucsc.edu, March 2006) and Ensembl Genome Browser (http://www.ensembl.org, February 2009). We sequenced candidate genes CACNB4 (calcium channel voltage-dependent subunit beta 4) and KCNJ3 (G-protein-activated inward rectifier potassium channel 1), which encode ion channels and are located on chromosome 2q. We performed PCR for all exons and splice junctions. We designed flanking exon primers from published sequences with Primer3 (ver. 0.4.0) online program (http://frodo.wi.mit.edu/primer3/input.htm). Primers sequences and PCR conditions are available on request. PCR products were purified with QIAquick PCR Purification Kit (Qiagen, Germantown, MD, USA) and sequenced with an ABI PRISM 3100 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA).

Results

Clinical description

A consanguineous Tunisian family segregating JME was identified (Fig. 1), conforming to the classification of the International League Against Epilepsy (ILEA) (Commission on Classification and Terminology of the International League Against Epilepsy, 1989). The parents are first cousins in a family that has lived in the region of Sidi-Bouzid in the Center of Tunisia for several generations. All the family members were directly interviewed by the same clinician (Dr. Mohamed Dogui). All affected sibs (patients IV-1, IV-4, IV-5, and IV-6) presented bilateral, irregular and arrhythmic myoclonic jerks involving the upper extremities that occur upon or shortly after awakening and often precipitated by sleep deprivation. These myoclonic jerks are often sustained by generalized tonic—clonic seizures (GTCS). Absences were not reported.