Juvenile Myoclonic Epilepsy Is Not Associated with the DRPLA Gene in a European Population

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Abstract. Background: Juvenile myoclonic epilepsy (JME), is an early-onset inherited generalized epilepsy which displays genetic heterogeneity, with at least 10 known loci. Another neurogenetic disease, dentato-rubro-pallido-luysian atrophy (DRPLA) presents three clinical phenotypes, one of which in Japanese displays many similarities to JME. Aim: The purpose of this study was to investigate whether the DRPLA gene is associated with JME in Caucasians. Results: The repeat sizes of all studied individuals were within the normal range. Discussion: These results seem to exclude the DRPLA gene as a major candidate gene for JME in this European population.

The spectrum of epilepsy types varies wildly and the prognosis for different entities is quite diverse. Among the epileptic syndromes in which the main feature includes myoclonic seizures, juvenile myoclonic epilepsy (JME) is regarded as the most common and well-delineated entity, affecting about 26% of all individuals with idiopathic generalized epilepsy (1). JME is an early-onset inherited type of generalized epilepsy (2-4). It is distinguished from other generalized epilepsies by its onset in early adolescence and the observation of myoclonic jerks, which are not necessarily a prelude to a major seizure and usually occur in the morning, upon awakening (3, 5). The presentation of all JME cases seems to belong in a defined clinical spectrum according to the diagnostic criteria of the International League Against Epilepsy (6, 7).

Nevertheless, JME displays genetic heterogeneity, with at least 10 loci identified so far, on chromosomes 6p21, 15q14, 2q23.3, 5q34, 1p36.33, Xp11.3, 5q12-q14, 6p12.2, 3q27.1, and 2q33-q36 (8-17). Very few patients with JME have been found to have recessive mutations which have been reported in four genes encoding calcium channel voltage-dependent beta-4 subunit (CACNB4) on 2q23.3, gamma-aminobutyric acid A receptor alpha-1 (GABRA1) on 5q34, gamma-aminobutyric acid A receptor delta (GABRD) on 1p36.33, and chloride channel 2 (CLCN2) on 3q27.1 (10-12, 16). Since JME is characterized by genetic heterogeneity with multiple involved genes with seemingly limited contribution to pathogenesis, there might also be other contributing genes.

One such gene might be the dentato-rubro-pallido-luysian atrophy (DRPLA) gene, which resides on 12p13 (18,19). In this gene, a trinucleotide repeat expansion causes dentato-rubro-pallido-luysian atrophy (MIM 125370), the juvenile type of which displays clinical similarities to JME (20, 21). A (CAG)n repeat, which is found in 7-23 copies in the normal population, is expanded to more than 48 repeats in patients with autosomal dominant neurodegenerative DRPLA (18,19). DRPLA is considered extremely rare in Europeans, but it is much more common in Japanese (18-25). The disease presents three clinical phenotypes (20, 26) associated with age of onset: late adult (ataxia, choreoathetosis and dementia but no epilepsy), early adult (similar to late adult, including epilepsy), and juvenile (initially myoclonus
epilepsy, but later ataxia, dementia or pyramidal signs may occur). The observed clinical phenotype is related to length of the expanded trinucleotide repeats.

Since in Japanese patients the juvenile type of DRPLA displays similar phenotypic features and age of onset to JME, the question is whether the (CAG)n repeat in the DRPLA gene might be associated with this myoclonic epileptic syndrome, at least in a subset of Europeans with JME. There are several paradigms of allelic mutations within a gene which cause different disorders with some common features. For example, point mutations in the calcium channel alpha-1A isoform 4 (CACNA1A) gene may cause either familial hemiplegic migraine or episodic ataxia type 2, while a (CAG)n repeat expansion in the same gene is associated with spinocerebellar ataxia type 6 (27). Interestingly, the variable length of a trinucleotide repeat may also be associated with different disorders, as in the case of DRPLA and autosomal recessive spastic paraplegia (18, 19, 28). The latter is caused by homozygosity of intermediate-size alleles of the (CAG)n repeat in the DRPLA gene.

In order to investigate whether the (CAG)n repeat in the DRPLA gene might be associated with JME, at least in a subset of Europeans, we examined this repeat with standard molecular techniques in Greek patients with JME. In addition, we examined some of their healthy relatives and healthy controls from the Greek population.

Materials and Methods

Blood samples were collected from 107 individuals of Greek origin, after informed consent. The studied individuals included 32 patients with JME (24 with sporadic and 8 with familial disorder), 25 of their healthy relatives (parents, brothers and sisters) and 50 healthy controls. The diagnosis of JME was established at University Departments of Neurology (Athens and Thessaloniki) and of Pediatrics (Athens), based on the patients’ clinical profile, history and electroencephalogram findings (EEG), according to specifically described inclusion/exclusion criteria of the International League Against Epilepsy (29).

The age of the patients ranged between 14-34 years (median=21 years) and the age at onset of the disease was 11-18 years (median=15 years). Regarding their gender, 26 (81%) were female and six (19%) were male. Most cases were sporadic (N=24), while in four families there were two affected children born to normal parents, suggesting autosomal recessive inheritance.

The relatives of the patients with JME were free of idiopathic epilepsy or other neurological disorder, had normal EEG spikes and their age ranged from 11 to 54 years (median=40 years). The controls were free of any neurological disorder and their age varied from 25 to 62 years (median=43 years).

Total DNA was extracted from blood samples using a standard NaCl technique. The polymorphic (CAG)n repeat of the DRPLA gene was studied using standard molecular methodology, as previously described (19). Briefly, the region containing the repeat was amplified using primers (VBC Genomics, Wien, Austria) and radioactive polymerase chain reaction (PCR) conditions and the PCR products were separated lengthwise by 6% polyacrylamide gel electrophoresis. A phage M13 sequencing ladder was electrophoresed in parallel as a marker in order to accurately determine the size of the (CAG)n repeat alleles. After vacuum drying of the gel, the radioactive bands were revealed by autoradiography.

Results and Discussion

The observed sizes of the (CAG)n repeat alleles of the studied healthy Greeks (9-20 copies, median=15, N=100) were in a range comparable to other studied European and Asian populations (18, 19, 22, 23, 30). The repeat alleles of the patients and their relatives were all in the normal range, as defined by the studied normal Greek population. The patients had 9-20 repeats (median=16, N=64), while their relatives had 9-19 repeats (median=15, N=50).

In conclusion, there was no indication of an expanded or an intermediate size of alleles in our sample of patients with JME. In addition, in two of the studied families, two patients with JME had the same two (CAG)n alleles as their healthy brothers. We consider this finding as evidence against linkage of JME and DRPLA.

JME is considered to be a phenotype included in the spectrum of idiopathic generalized epilepsy, which is a common complex disease with an almost exclusively genetic etiology, but with variable phenotypes (31). Varying combinations of several interacting genes may result in clinical phenotypes such as juvenile absence epilepsy, epilepsy with generalized tonic clonic seizures, or JME. In support of this hypothesis, the same cys104phe mutation in the CACNB4 gene has been found in patients with diverse phenotypes, such as rare juvenile atypical prolonged absences with occasional tonic-clonic seizures, and episodic ataxia with recurrent episodes of vertigo, truncal ataxia and dysarthria (10).

Therefore, it seems that the clinical features of myoclonic jerks and ataxia may be included in the clinical spectrum of both idiopathic generalized epilepsy and DRPLA. The two disorders share some clinical similarities, possibly due to overlapping molecular pathogenic mechanisms. Interestingly, it is well documented that the signal transduction pathways involving both the DRPLA protein and JME-related ion channels CACNB4 and CLCN2 lead to activation of the transcription factor cAMP-response element-binding protein (CREB) (32-34).

In the present study, we investigated the hypothesis that the (CAG)n repeat might be associated with JME at least in a subset of Europeans. Our results provide evidence for exclusion of the DRPLA gene as a major candidate gene for juvenile myoclonic epilepsy in Europeans. Additional studies of more DNA samples of Caucasian patients with JME are necessary in order to strengthen this point beyond any reasonable doubt.
References


