

## Absence of *GABRA1* Ala322Asp mutation in juvenile myoclonic epilepsy families from India

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### Abstract

An Ala322Asp mutation in the *GABRA1* gene was recently reported to be responsible for causing the autosomal dominant (AD) form of juvenile myoclonic epilepsy (JME) in a French-Canadian family. To study if JME families from India exhibiting the AD mode of inheritance carry the Ala322Asp mutation, we examined 35 unrelated JME-affected individuals from such families for the Ala322Asp mutation in *GABRA1*. Ala322Asp mutation was not observed in any of these JME-affected individuals, suggesting that this mutation is unlikely to be a predominant mutation involved in causation of epilepsy. To evaluate the possibility of other mutation(s) in and around *GABRA1* that may predispose to JME, we compared the allele frequencies at two marker loci, D5S2118 and D5S422, flanking *GABRA1*, in probands and 100 matched population controls. One of the allele frequencies at D5S422 shows a significant difference between the cases and controls ( $\chi^2 = 11.44$ , d.f. = 1,  $P = 0.0007$ ), suggesting genetic association between JME and genes located in the proximity of the DNA marker.

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### Introduction

Juvenile myoclonic epilepsy (JME) is a clinically well-defined, age-related idiopathic generalized epilepsy (IGE) syndrome, which accounts for about 10% of all epilepsies (Janz and Durner 1997). JME develops around adolescence and is characterized by bilateral, single or repetitive arrhythmic irregular myoclonic jerks, predominantly in arms and shoulders. Evidence for substantial hereditary contribution to the aetiology of JME is well established (Janz *et al.* 1989). JME is genetically heterogeneous. Four genetic loci predisposing to JME have been found. These map at

6p21.3 (Greenberg *et al.* 1988; Durner *et al.* 1991; Weissbecker *et al.* 1991; Greenberg and Delgado-Escueta 1993; Sander *et al.* 1997; Greenberg *et al.* 2000), 6p12-11 (Liu *et al.* 1995, 1996), 6q24 (Bata *et al.* 2000) and 15q14 (Elmslie *et al.* 1997). However, mutational analyses of candidate genes *LRRC1* (leucine-rich-repeat-containing 1), *GCLC* (glutamate-cysteine ligase, catalytic subunit), *KIAA0057* (translocation-associated membrane protein 2) and *CLIC5* (chloride intracellular channel 5) from 6p11-12 have not identified disease-causing mutations (Suzuki *et al.* 2002). Similar studies on candidate genes *CHRNA7* (cholinergic receptor, nicotinic, alpha polypeptide 7) (Taske *et al.* 2002) and *KCC3* (solute carrier family, member 6) (Steinlein *et al.* 2001) on 15q14 have not revealed disease-causing variants.

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Cossette *et al.* (2002) recently made an interesting finding in the area of JME genetics. An Ala322Asp mutation in the *GABRA1* gene that codes for the  $\alpha 1$  subunit of the  $\gamma$ -aminobutyric acid receptor subtype A (GABA<sub>A</sub>, chromosomal location 5q34) was found in the affected members of a large French-Canadian family exhibiting the autosomal dominant form of JME. This mutation in *GABRA1* is the first known mutation that segregates with the classical JME clinical phenotype. All affected individuals in this family were heterozygous for a C → A substitution in exon 9 of *GABRA1*, which is predicted to change a GCC (alanine) to a GAC (aspartic acid) codon at position 322 of its cDNA. GABA<sub>A</sub> receptors are ligand-gated chloride channels, which mediate fast synaptic inhibition in the brain. HEK293 cells expressing the mutant GABA<sub>A</sub> receptor ( $\alpha 1_{\text{Ala322Asp}} \beta 2 \gamma 2$ ) have lower amplitude of GABA-activated currents compared to those expressing the wild-type ( $\alpha 1 \beta 2 \gamma 2$ ) receptor (Cossette *et al.* 2002), indicating that seizures may result from loss of function of this inhibitory ligand-gated channel.

To study the role of *GABRA1* in JME, we have screened for presence of the Ala322Asp mutation in JME families exhibiting dominant inheritance from India. We have genotyped probands and control population for two DNA markers flanking *GABRA1* to find out the possibility of genetic association. Results of these studies are presented here.

## Materials and methods

**Subjects:** The study was performed on unrelated JME probands from families with at least one parent affected with JME or a subtype of IGE. All individuals were diagnosed according to the guidelines based on the Commission on Classification of Epilepsies and Epileptic Syndromes of the International League Against Epilepsy (ILAE) (1989). A total of 35 families were collected upon informed written consent. Electroencephalographic (EEG) recordings were taken for all index cases ( $n = 35$ ). Clinical details of affected individuals are summarized in table 1. Ten ml of peripheral venous blood was collected from all the participants and genomic DNA was extracted using the phenol–chloroform method (Sambrook *et al.* 2001).

***GABRA1* Ala322Asp mutation screening:** Exon 9 of *GABRA1* was amplified from genomic DNA using intronic primers 5'TGCCATTCCATGAATCACAG3' and 5'TCATGGCACTTAATTGTTTACG3'. PCR was performed with 150 ng genomic DNA, 25 pmol primers, 10 mM Tris-Cl, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 800  $\mu$ M dNTPs and 2.5 U *Taq* DNA polymerase (Gibco-BRL) in a total reaction volume of 50  $\mu$ l for 40 cycles (each cycle of 94°C for 30 s, 55°C for 30 s and 72°C for 60 s). The 543-bp PCR product was purified using Qiaquick columns (Qiagen) and cycle sequencing was performed using 30 ng of the purified PCR product, 3.2 pmol of each primer and 4  $\mu$ l of the Big Dye<sup>TM</sup> Terminator v3.0 Cycle Sequencing Ready Reaction mix (Applied Biosystems) in a total reaction volume of 10  $\mu$ l for 25 cycles (each cycle of 96°C for 10 s, 50°C for 5 s, 60°C for 4 min). Following cycle sequencing, the samples were purified and precipitated with ethanol, resuspended in Hi-Di Formamide (Applied Biosystems), denatured at 95°C for 5 min, and loaded onto an ABI 3100 Genetic Analyzer (Applied Biosystems/Hitachi). The sequence of each amplicon was confirmed by sequencing in both directions. Sequences were compared with the reference *GABRA1* mRNA sequence (GenBank NM\_000806). Alignments and analysis were performed using ClustalX version 1.8 (<http://www.molbiol.ox.ac.uk/documentation/clustalx/clustalx.html>).

**Genotyping:** Two markers, D5S2118 (AFMa102zg9) and D5S422 (AFM211yc7), flanking *GABRA1*, were used to genotype 35 JME probands and 100 matched population controls. D5S2118 is located ~ 500 kb upstream of *GABRA1* and D5S422 is located ~ 800 kb downstream. PCR was performed with 50 ng genomic DNA, 2.5 pmol primers, 10 mM Tris-Cl, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 800  $\mu$ M dNTPs and 0.5 U *Taq* DNA polymerase (Gibco-BRL) in a total reaction volume of 10  $\mu$ l for 40 cycles (each cycle of 94°C for 30 s, 60°C for 30 s and 72°C for 30 s). PCR products were denatured with Hi-Di Formamide (Applied Biosystems) at 95°C for 5 min and loaded onto an ABI 3100 Genetic Analyzer (Applied Biosystems/Hitachi). Genotyping analysis was done using Genotyper version 3.7 (Applied Biosystems).

**Table 1.** Clinical details of JME-affected individuals.

Total number of individuals	35	
Mean ( $\pm$ S.D.) age of onset (years)	15.4 ( $\pm$ 3.7)	
	Myoclonic jerks only	4
Number of probands with a particular type of seizure(s)	Myoclonic jerks + generalized tonic–clonic seizures	26
	Myoclonic jerks + absences	1
	Myoclonic jerks + generalized tonic–clonic seizures + absences	4
Number of individuals with generalized polyspike-and-wave discharges	33	
Number of individuals with normal EEG	2	

## Results and discussion

A total of 35 unrelated JME probands were studied. Mean age of onset of seizures was 15.4 years. The majority of affected individuals (26 out of 35) had myoclonic jerks and generalized tonic-clonic seizures (GTCS); four affected individuals had myoclonic jerks, absences and GTCS. An additional four cases had only myoclonic jerks and there was one case that exhibited myoclonic jerks and absences. Thirtythree probands had EEG abnormalities and the remaining two had normal EEG recordings. *GABRA1* Ala322Asp mutation was not observed in any of the JME-affected individuals studied. Sequence alignments and comparisons with the wild-type sequence (GenBank NM\_000806) did not indicate any other coding or splice-site variant in exon 9. Ala322Asp mutation in *GABRA1* is located in a highly conserved domain of the protein. Ala322 residue is located in the third transmembrane domain of the predicted protein and is conserved in GABA<sub>A</sub>  $\alpha$ 1 subunit genes from different species. Ala322 is also conserved in the other five known human GABA<sub>A</sub>  $\alpha$ -subunit genes, *GABRA2*, *GABRA3*, *GABRA4*, *GABRA5* and *GABRA6*. Absence of *GABRA1* Ala322Asp mutation in

the present study suggests that it is a rare mutation predisposing the particular family studied by Cossette *et al.* (2002) and is unlikely to be a common cause of JME in families from India. But the results are similar to other findings by Cossette *et al.* (2002), where they did not find this Ala322Asp variation in 31 sporadic JME cases of French-Canadian origin.

Even though Ala322Asp mutation is absent in the sample studied, there is a possibility that additional mutation(s) at *GABRA1* may predispose to JME. To evaluate this possibility we compared the allele frequencies at two marker loci flanking *GABRA1* in probands and normal controls. Significant differences in allele frequency at marker loci between cases and controls are considered to be suggestive of genetic association. Dinucleotide CA-repeat markers D5S2118 and D5S422 located close to *GABRA1* were genotyped in 35 JME probands and 100 control individuals. The results of genotyping are summarized in table 2. A total of six alleles were observed at D5S2118 with allele size range of 241–261 bp. Allele with size 241 was not observed in the control sample. Allele with size 253 was the most common in both cases and controls. No significant difference in allele frequencies was observed

**Table 2.** Allele frequency comparisons of JME-affected individuals and controls at markers D5S2118 and D5S422.

### Marker D5S2118

Controls ( <i>n</i> = 100)				Cases ( <i>n</i> = 35)			
Allele size	Frequency	Allele size	Frequency	Observed counts	Expected counts	$\chi^2$ (1 d.f.)	<i>P</i> value
241	0	241	0.043	3	0	–	–
251	0.045	251	0.029	2	3.15	0.419	0.517
253	0.525	253	0.528	37	36.75	0.002	0.964
255	0.330	255	0.3	21	23.1	0.191	0.662
257	0.015	257	0.290	2	1.05	0.859	0.354
261	0.085	261	0.071	5	5.95	0.152	0.697

### Marker D5S422

Controls ( <i>n</i> = 100)				Cases ( <i>n</i> = 35)			
Allele size	Frequency	Allele size	Frequency	Observed counts	Expected counts	$\chi^2$ (1 d.f.)	<i>P</i> value
112	0.075	112	0.186	13	5.25	11.440	0.0007
114	0.005	114	0.029	2	0.35	7.778	0.005
120	0.280	120	0.186	13	19.6	2.222	0.136
122	0.040	122	0.043	3	2.8	0.014	0.906
124	0.020	124	0	0	1.4	1.4	0.237
126	0.020	126	0.043	3	1.4	1.828	0.176
128	0.180	128	0.143	10	12.6	0.536	0.464
130	0.155	130	0.157	11	10.85	0.002	0.964
132	0.190	132	0.186	13	13.3	0.007	0.933
134	0.030	134	0.029	2	2.1	0.005	0.944
136	0.005	136	0	0	0.35	0.35	0.554

at this locus. A total of 11 alleles were observed at D5S422 with allele size range of 112–136 bp. Alleles with size 124 and 136 were not observed in cases. Alleles with size 112, 120 and 132 were the most common (frequency = 0.186) and alleles with size 114 and 134 were the least common (frequency = 0.029) among the cases. Allele with size 112 was more common in cases compared to controls ( $\chi^2 = 11.44$ , d.f. = 1,  $P = 0.0007$ ). This difference remains significant even after correction for multiple testing (corrected  $\alpha$  value 0.0045; 0.05/11). These results are suggestive of a genetic association between JME and genes located in proximity of D5S422. Other than *GABRA1*, the genomic region close to D5S422 also harbours two other GABA-family genes, namely *GABRA6*, which encodes the  $\alpha 6$  subunit of  $\gamma$ -aminobutyric acid receptor subtype A, and *GABRG2*, which encodes the  $\gamma 2$  subunit of  $\gamma$ -aminobutyric acid receptor subtype A. This makes these genes good candidates for screening for mutation(s) that may underlie JME phenotype.

Epilepsy is associated with hypersynchronous activation of large populations of neurons. There has been considerable interest in the possibility that disinhibition of 'GABA' activity is associated with the pathogenesis of some forms of epilepsy. That modified GABAergic inhibition produces epileptiform discharge has been based on the experimental evidence that drugs which block GABAergic inhibition produce paroxysmal bursting in isolated neurons (Schwartzkroin and Prince 1980). Application of GABA antagonists produces large depolarization because of combined giant excitatory postsynaptic potentials, thus producing paroxysmal depolarization shifts (PDS), which are a manifestation of an epileptic event. Production of PDS requires a combination of reduced GABAergic inhibition, feed-forward excitation and bursting properties of individual neurons (Traub and Wong 1982). Although *GABRA1* Ala322Asp mutation is the very first example of a large human family where a *GABRA1* mutation is shown to cause a common epileptic syndrome, this mutation does not appear to account for the large number of dominantly inherited JME cases, as shown by our results as well as other findings reported by Cossette *et al.* (2002). However, these results do provide suggestive evidence for a possible genetic association between JME and a different mutation in the same genomic region that harbours a number of genes encoding additional subunits of GABA<sub>A</sub> receptor.

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