



## **Assessment of Gene Polymorphism in GABAA1 Receptor among Sudanese Patients with Juvenile Myoclonic Epilepsy (JME)**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Introduction:** Juvenile myoclonic epilepsy (JME) is an idiopathic generalized epilepsy syndrome (IGE) with a strong genetic contribution. The main characters of JME are generalized convulsive or an absences seizures proceeded by myoclonic jerks. Gene variant of gamma-aminobutyric acid type A inhibitory receptor speculated to underlay JME etiology.

**Objective:** This study aimed to screen for JME based on the International League against Epilepsy Commission on Classification and Terminology diagnostic criteria and to assess the link of polymorphism in the GABAA receptor gene, GABRA1 to the development of JME in Sudanese patients.

**Methods:** Our epidemiological study enrolled 44 JME patients, only 23 participated in the genetic part and 35 matched healthy controls were also included. Blood genomic DNA was isolated and PCR based- restriction fragment length polymorphism (RFLP) analysis was done. The data obtained were analyzed using computer software SPSS 21rt edition.

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**Results:** The frequency of the mutant G allele was found to be 41.5% in patients and 45.5% in the controls. The genotype distribution of A and G alleles among patients were found to be (AA= 39%), (AG=39%) and (GG= 22%) and that of the controls were (AA=40%), (AG=30%) and (GG= 30%).

**Conclusion:** The mutant G allele of the GABAA1 receptor does not affect the development of JME in Sudanese patients but the AG genotype may be a risk factor.

*Keywords: Gene Polymorphism; GABAA1, JME; epileptic syndrome.*

## 1. INTRODUCTION

It is reported that Juvenile myoclonic epilepsy represents 10% of all idiopathic generalized epilepsies, the disease is characterized by jerks and generalized tonic-clonic seizures (GTCS). The instability of neural networks caused by ion channel abnormalities is generally assumed to represent the basis of the pathophysiology of JME, abnormal cerebral structure and function mainly involving the frontal lobes have been reported [1]. Various heritability models have been used to clarify the genetic basis of JME, those models include major Mendelian inheritance or concurrent contribution of multiple genes with minor effects in non-Mendelian fashion [2]. Based on genetic epidemiological studies the classical JME appears to have complex genetic traits caused by interaction of several genetic and environmental risk factors [3]. A range of (17- 49)% of patients with JME have relatives with different epileptic seizures [4]. The risk of having clinically evident JME is small and the disease burden varies across spectrum of individuals. Many studies have presumed that JME is an autosomal dominant disease of incomplete penetrance which means that some individuals who inherit JME genes do not express clinical JME. However, their children may inherit the clinically phenotyped syndrome [5,6,7]. It is reported that monogenic forms of IGEs are associated with various mutations in genes encoding for voltage and ligand-gated ion channels including a mutation in type A gamma-aminobutyric acid (GABAA) receptor genes [8,9]. The instability of neural networks caused by ion channel abnormalities is assumed to represent the basis of the pathophysiology of JME [9,10]. Among JME patients-based studies, the extensive existence of central nervous system GABA inhibitory genetic abnormalities acquired an enormous concern [11]. GABAA receptors are ligand-gated chloride channels and their molecular structure comprises a heteropentameric protein complex assembled from 19 different classes of subunits. Epilepsy-causing mutations have been identified in GABRA1 [8,12] and GABRG2 [13,14,15].

Several single nucleotide polymorphisms (SNPs) in the GABAA receptor have been described but only the intronic GABRA1 IVS11 + 15 A > G and the exonic GABRG2 588C > T gene polymorphisms are found to have functional significance in different neurological disorders. These gene variants have been attributed to several susceptibility factors for febrile seizures [16,17]. The mutant receptors have low affinity for GABA and they showed reduced chloride current amplitude when they fully saturated compared to wild-type receptors. Both inactivation and re-activation kinetics were significantly enhanced in mutant channels. In at least two mutations in GABRG2 [13,15] and two mutations in GABRA1 [12,18] has shown the reduction in the amplitude of GABA-evoked current due to reduced surface expression of the receptor protein caused by retention of the mutant receptors in the endoplasmic reticulum [18]. This is consistent with loss of function mutations found in at least four different subunits of the GABAA receptor for related epileptic syndromes. These suggest impairment in the degree of inhibition mediated by GABAA receptors in the pathophysiology of the classical IGE with autosomal dominant inheritance. High genetic heterogeneity has been observed in early linkage studies on JME, it is likely that additional ion channel genes will be involved in JME. Genes encoding GABAA receptor subunits come at the top of the list of candidates for epilepsy susceptibility [19]. Based on functional significance, previous observations and current knowledge, in our genetic study we investigated the possible link of the GABRA1 IVS11 + 15 A > G (rs2279020) polymorphism with susceptibility to juvenile myoclonic epilepsy among Sudanese patients.

## 2. MATERIALS AND METHODS

### 2.1 Patients and Controls

This research study consisted of two parts, an epidemiological and a genetic study. The proposal of the study was approved by the Ethical Research Committee of the National

**Table 1. Primers and restriction enzymes used for amplification of GABRA1 (rs2279020)**

SNP	Primer sequence	Restriction enzyme	Reference
GABRA1 IVS11 + 15 A > G (rs2279020)	F 5'-GCT ATG GAT TGG TTT ATT GCC GTG TG-3' R 5'-ATA ATA TTG ATG TAC TAC AGG GAC-3'	Avall	(20)

Ribat University in Khartoum. All participants or their legal guardians signed a written informed consent disclosing their rights and confidentiality guarantees before starting the study procedures. All the study subjects were residents of Khartoum state, the capital of Sudan. The epidemiological study population consisted of 3523 unrelated patients attended to the EEG unit of the Faculty of Medicine, National Ribat University and M.S Elmagzoub Neurosciences Center in Khartoum State (March 2003 to May 2012). The diagnosis of JME is based on the International League against Epilepsy Commission on Classification and Terminology diagnostic criteria. Forty-four patients were diagnosed as JME patients based on their EEGs characteristic features (consisting of generalized 3.5-6 Hz single, bifid and polyspikes slow - waves complexes on normal brain background activity) and only twenty-three patients agreed to participate in the genetic study. The study also included 35 healthy controls that were matched with patients by age, gender, ethnicity and residence. The demographic data and subjects' clinical history were obtained using a predesigned interviewing questionnaire. None of the controls experienced epileptic seizures or had a neuropsychiatric disease. Three ml of venous blood samples were collected from patients and controls and were preserved at -20°C for the genetic analysis.

## 2.2 Genetic Analysis

### 2.2.1 Laboratory investigations

#### 2.2.1.1 DNA isolation and PCR-RFLP Genotyping of GABRA1 (rs2279020)

The gene variants were detected by methods described by Chou IC et al. [15] and Park Cs et al. [20]. Genomic DNA was isolated from peripheral blood leucocytes using the standard salting-out method with slight modifications [21]. GABRA1 (rs2279020) genotyping was performed using PCR-RFLP analysis (Table 1). PCR conditions were as follows: a denaturing step at 95°C for 5 min, then 30 cycles at 94°C for 30s, the annealing temperature is 60°C for 30s, then

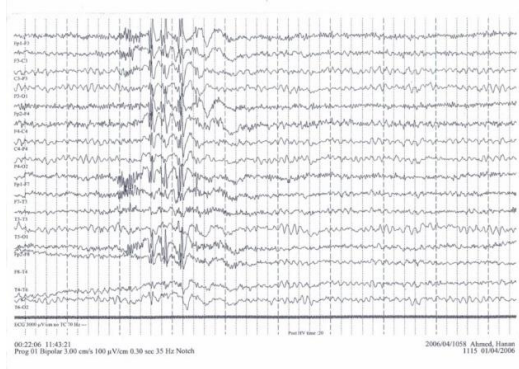
72°C for 30 s, and a final extension at 72°C for 7 min. PCR products of 165 bp was observed for GABRA1 (rs2279020) which was then digested using the specific restriction endonuclease Avall (Fermentas Inc., USA) RFLP assay in GABRA1 IVS11 + 15 A > G polymorphism was used to distinguish the A/G substitution at nucleotide 15 of the last intron and the products were separated on 2% agarose gel Figure. [3], the genotyping patterns were recorded as 'AA' homozygote showing a band with 165 bp, 'AG' heterozygote with three bands, 165 bp, 141 bp and 20 bp and the 'GG' homozygote with two bands, 141 bp and 20 bp. Gel documentation was done using Alpha Imager™ 1220, Alpha Innotech Corporation, and San Leandro, CA.

## 3. RESULTS

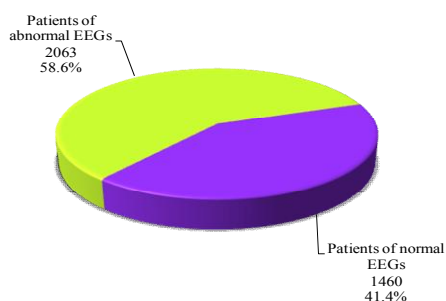
The study revealed that out of the 3523 patients attended to our clinics, 1460 (41.4%) had normal EEGs (Fig. 1) while 2063 (58.6%) patients had abnormal EEGs (Fig. 2), of those patients only 44(2.13%) their EEGs showed the characteristics electrical features of JME with moderate females' predominance (56.81%) females and (43.2%) males (Table 2). Absence attacks were confirmed in (77.27%) of JME patients and the generalized tonic-clonic convulsions GTCS reported in (84.1%) of patients, with a mean age of onset (13.92 ± 5.65) years (Table 3). Of the JME patients, 92% showed no aura symptoms and postictal sleepiness was confirmed in (75.7%) of them, but all patients lost their consciousness. The mean age of JME patients at diagnosis was (19.55 ± 8.98) years; (75%) of them were between 10 and 30 years. Myoclonic jerks were detected in (90.9%) of patients with a mean age of onset (10.48 ± 4.81) years. Sleep disturbance was found in (40.9%), memory capacities declined in (79.5%), and school or work performance deteriorated in (86.4%). Of JME patients (75%) had positive family history of epilepsy; of whom (66.3%) were first degree, while (33.7%) were second-degree relatives. The results of the genetic study showed that for the GABRA1 IVS11 + 15 A > G (rs2279020) polymorphism, the frequency of the A allele was found to be 41% in patients and 45% in controls

and that of G allele was 41% among patients and 45% among the control group. The genotype distribution of A and G alleles for patient were (AA= 39%), (AG=39%) and (GG= 22%) and that

of the controls were (AA=40%), (AG=30%) and (GG= 30%). The male: female ratio of patients was 2:2.5 and that of controls was 3:3.5 Table [2].



**Fig. 1. Generalized 3.5-6 Hz single, bifid and polyspikes – slow wave complexes on normal brain background activity**



**Fig. 2. Normal and abnormal EEGs in patients referred to National Ribat University and Elmagzoub Neurosciences Center (March 2003 to May 2012)**

**Table 2. The prevalence of JME among patients with abnormal EEGs**

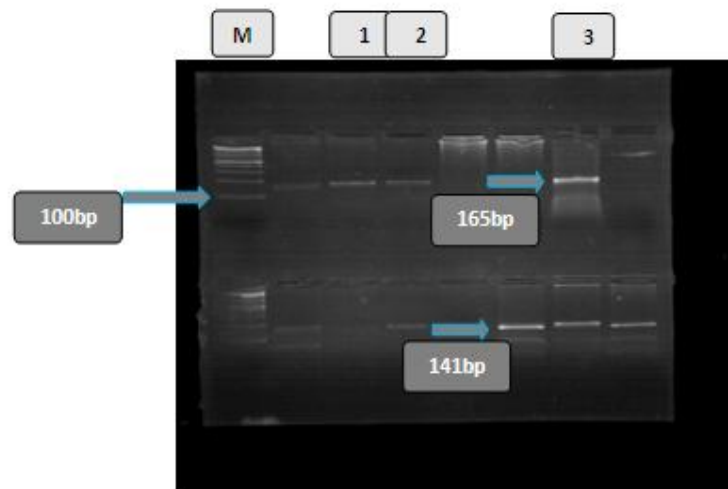
Patients with abnormal EEGs	Males No. (%)	Females No. (%)	Total No. (%)
Juvenile myoclonic epilepsy (JME)	(43.2%)	(56.81%)	44(2.13)
Patients not had JME			2019(97.87)
Total			2063(100)

**Table 3. Generalized and absence seizures in sudanese patients with JME**

Seizure	No.	%
1. Absence attacks	34	77.27
2. GTC	37	84.1
Tonic	4	9.1
Clonic	6	13.6
Tonic-clonic	34	77.3
Age of Onset of GTC (Years) (n=37)		
Min. – Max.	2.0 – 29.0	
Mean ± SD.	13.92 ± 5.65	
Median	14.0	

**Table 4. Allele frequency and genotype distribution of GABAA1 alleles among the study subjects**

<b>GABRA1 IVS11 + 15A &gt; G (rs2279020)</b>	<b>JME Patients</b>	<b>Controls</b>
Allele frequency:		
A	58%	55%
G	41.5%	45%
Genotype distribution:		
AA	39%	40%
AG	39%	30%
GG	22%	30%



**Fig. 3. RFLP analysis of GABRA1IVS11 + 15 A > G (rs2279020) on 2% agarose gel, M: DNA Ladder (100pb); Lane's 1-3 patients samples**

#### 4. DISCUSSION

At the time of our epidemiological study 44 (2.13%) of the patients were found to be typical cases of JME. Of those patients 77.3% showed the classical symptoms triad (jerks, absences and GTCS). This finding is inconsistent with Murthy et al. observation, who found it in 17.5 % of patients [22]. The significant distressing event for 84.1% of our patients was the generalized tonic-clonic convulsions; Ali et al. documented the GTCS in 94.1% of patients with JME [23]. In all Sudanese JME patients, the GTCS followed the MJs by 4.4 years compared to 3.3 years indicated by Asconape and Penry (1984). It was evident that most of our patients didn't come for specialized medical advice until they developed GTCS. As far as we know, this is the first study that assessed genetic polymorphism of GABRA1 (rs2279020) in Sub-Saharan African patients with JME. Our findings heightened the assumption of no relationship between G allele and JME phenotype. Genotyping disclosed the frequency

of the mutated G allele among patients and controls was so close, 41.5% and 45%, respectively. It is also similar to the results of the studies carried out by Kananura et al. [14] and Chou IC et al. [15]. This consensus has been scaffolded by the Bruna Priscila dos Santos et al. [3] meta-analysis outcomes. There is no significant association between IGEs and either A or G alleles by reviewing over 50 population-based genetic association studies investigating polymorphism with JME. But the inconsistency appeared when our results likened to Ritu Kumari et al. [24], who found that the muted G allele was significantly high in Indian patients with IGEs. This discrepant outcome might be due to our small sample size and also the effect of other environmental and genetic factors that supporting the speculation of non-Mendelian heredity. Considering the general population, including both patient (P/C) control individuals, the prevalence of homozygous and heterozygous carriers was AA (39% / 40%), AG (39% /30%), and GG (22%/30%). Showed no significant

difference except for the AG genotype, which is higher by 10% in patients. These findings agree with the results obtained in India and China by Kananura et al. and Chou IC et al, respectively [14-15].

## 5. CONCLUSION

The prevalence of JME among Sudanese epileptic patients is not different from international reports with clues of family's occurrence. The mutant G allele of the GABAA1 receptor gene do not affect the development of JME in Sudanese patients. The AG genotype of GABAA1 receptor may be a risk factor for JME in Sudan.

## 6. LIMITATIONS AND RECOMMENDATIONS

We recommend a study with a large sample size, including detailed clinical investigations and genetic analysis of other subunits of GABA receptor, to demonstrate the role of the family history in the disease's occurrence in Sudan and to confirm our results.

The culture of research participation among the Sudanese population is feeble, particularly regarding their genetic research. More than half of our initial recruited patients (44 patients) refused to participate in the genetic analysis.

## CONSENT AND ETHICAL APPROVAL

The proposal of the study was approved by the Ethical Research Committee of the National Ribat University in Khartoum. All participants or their legal guardians signed a written informed consent disclosing their rights and confidentiality guarantees before starting the study procedures.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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