ORIGINAL ARTICLE

Association of rs211037 GABRG2 gene polymorphism with susceptibility to idiopathic generalized epilepsy

Marija Milanovska[1,2], Emilija Cvetkovska[1], Sasho Panov[3]

[1]University Clinic of Neurology, Saints Cyril and Methodius University, [2]Neuromedica Hospital, [3]Department of Molecular Biology and Genetics, Faculty of Sciences, Saints Cyril and Methodius University; Skopje, Republic of North Macedonia

ABSTRACT

Aim This case-control study aimed to determine a possible association of single nucleotide polymorphism rs211037 of the gamma-aminobutyric acid receptor subunit gamma-2 (GABRG2) gene with the susceptibility to idiopathic generalized epilepsy (IGE) in the Macedonian population.

Methods It enrolled 96 patients with clinically verified IGE and 51 healthy individuals without personal and family history of epilepsy or other neurological disorders as controls. A determination of the GABRG2 rs211037 polymorphism was performed using the TaqMan-based genotyping assay.

Results A significant dominant association of the CC genotype (odds ratio - OR=2.100, 95% CI=1.018-4.332; p=0.043) and allelic association of C allele (OR=1.902, CI=1.040-3.477; p=0.035) with susceptibility to IGE was found. Carriers of CC genotype had approximately a 2-fold higher probability of developing IGE than the carriers of CT and TT genotypes. Carriers of the C allele had a 1.9-folds higher probability for IGE than the carriers of the T allele.

Conclusion The polymorphism rs211037 of the GABRG2 gene increases the risk of the development of idiopathic generalized epilepsy in the Macedonian population.

Key words: case control studies, epilepsy, GABA receptors, single nucleotide polymorphism

Corresponding author:
Emilija Cvetkovska
University Clinic of Neurology
Vodnjanska str. 17, MK-1000 Skopje,
Republic of North Macedonia
Phone: +389 70 338 372;
E-mail: emilicvetkovska@gmail.com
Marija Milanovska ORCID ID: https://orcid.org/ 0000-0003-1652-4661

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INTRODUCTION

The gamma-aminobutyric acid type A ($GABA_A$) receptors are the major inhibitory neurotransmitter receptors in the mammalian brain and are crucial in controlling the activity of neuronal networks (1,2). The $GABA_A$ receptor is a pentameric chloride ion channel and consists of two α, two β, and one γ subunits, encoded by the gamma-aminobutyric acid type A receptor subunit alpha1 (GABRA1), gamma-aminobutyric acid type A receptor subunit beta2 (GABRB2), and gamma-aminobutyric acid type A receptor subunit gamma2 (GABRG2) genes, respectively, and with the most common subunit composition being α1β2γ2 (1,3). Among epilepsy-associated mutations or variants in different $GABA_A$ subunits, a substantial amount is found in the GABRG2 gene and they are connected with a variety of seizures and epilepsy types from self-limiting febrile seizures (FS) to drug-resistant epilepsies with comorbidities and epileptic encephalopathies (4-7).

GABRG2 gene is located on the long arm of chromosome 5 (5q34). The rs211037 single nucleotide polymorphism (588C>T or Asn196Asn) in exon 5 on the GABRG2 gene results in a synonymous variant allele that may change its expression affecting the transcription, mRNA stability, abnormal subunit folding, as well as aberrant glycosylation and translation efficiency of the GABRG2 receptor synthesis. Consequently, the receptor response to extrinsic environmental signals may be altered by still unknown mechanisms (8). The results of the studies that have examined exonic GABRG2 rs211037 locus have been inconsistent and the frequency of variants seems distinctive in different ethnical groups (9-13). A few of them shed light on its possible link with susceptibility to febrile seizures (FS) and idiopathic generalized epilepsy (IGE) specifically (14-15).

Given the reported variance in allele frequencies and susceptibility to IGE between diverse populations, as well as lack of similar genetic investigation in the Macedonian population, we performed a case-control study aimed to explore the possible association of GABRG2 rs211037 polymorphism with the susceptibility to IGE.

PATIENTS AND METHODS

Patients and study design

Consecutive 96 patients aged ≥14 years with idiopathic generalized epilepsies (IGE) attending the Epilepsy Outpatient Clinic of a University Hospital for Neurology in Skopje from March 1 2019 to February 28 2020 were enrolled. Patients (IGE group) were eligible for the study if they were meeting the criteria of the International League Against Epilepsy (ILAE) guidelines for the classification of seizures and epilepsy syndromes (16). Specifically, we included patients with four well-established IGE syndromes: childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy (JME), and generalized tonic–clonic seizures alone (GTCS alone). The diagnosis was made on clinical grounds, supported by the finding of generalized spike and wave or polyspike and wave pattern in EEG.

We routinely performed 20-minute awake EEG recording with standard activation procedures (hyperventilation and photostimulation). If the initial EEG assessment was normal or inconclusive, we proceeded with a prolonged (2-hours) EEG, which was done in the morning after whole night sleep-deprivation. Patients with normal sleep-deprived EEG were not included in the study, because of a lower degree of diagnostic certainty for IGE. Patients with lesional MRI were excluded from the study, too. The patients with CAE were originally diagnosed and treated at Children's hospital, and then because their seizures persisted after the age of 14 years, they continued their treatment at the Clinic for Neurology, where they were recruited for the study.

General information such as age, sex, prenatal and perinatal history, intellectual and motor development as well as data regarding the history of epilepsy i.e. age of onset and seizure types, family history, treatment, and seizure control was collected.

Further, 51 age- and gender-matched healthy individuals from the general population were recruited as non-epilepsy controls. Subjects with personal or family history of epilepsy or any other neurological disorders were excluded from the study.

The Ethics Committee of the Medical Faculty at the of Saints Cyril and Methodius University in

Skopje approved this case-control study and all participants signed informed written consents before participating in the study.

Methods

A venous blood sample (2-3 mL) was collected from each participant into an EDTA tube and the genomic DNA was extracted using PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific) and the extracted DNAs were stored at -20°C. Determination of the GABRG2 rs211037 polymorphism was performed using TaqMan fluorescence probes-based real-time polymerase chain reaction amplification genotyping (Thermo Fisher Scientific, Thermo Fisher Scientific, Waltham, MA, USA) on a StepOne RT-PCR System (Applied Biosystems, Applied Biosystems, Foster, CA, USA) according to the manufacturer's instructions. The genotype calling was performed based on the allele-specific fluorescence by allelic discrimination utility of the StepOne software included in the system.

Statistical analysis

Genotype and allele frequencies of the GABRG2 rs211037 polymorphism were determined by allele counting. $\chi 2$ test or Fisher's exact test (two-tailed, when the subgroups had less than 5 samples) were used to estimate the significance of genetic association with susceptibility to IGE. The odds ratio (OR), as well as the corresponding 95% confidence interval (95% CI) were calculated to evaluate polymorphism association with the probability-susceptibility to IGE. The association was considered significant when the p-value was <0.05. Hardy-Weinberg equilibrium was calculated by the $\chi 2$ test. Statistical and population genetic analyses were performed by XLSTAT 2016 and GenAlEx 6.5 software.

RESULTS

The IGE patients and control participants had matched gender and were of comparable age structure. The JME was the most common syndrome presented in 52 (54%) patients (29 females and 23 males). Thirty-six (38%) patients fulfilled the criteria for GTCS alone (17 females and 19 males). Eight (8%) patients had absence epilepsy (five with JAE and three with CAE).

Table 1. Selected demographic and clinical characteristics of the patients with idiopathic generalized epilepsy (IGE) and controls

Characteristic	**IGE patients** (n=96)	**Controls** (n=51)
Gender (No; %)		
Males	47 (49 %)	27 (53 %)
Females	49 (51 %)	24 (47 %)
Age (mean ± SD) (range) (years)		
Age overall	32.6 ± 13.6 (14-78)	39.7 ± 6.7 (28-53)
Age at seizure onset	17.2 ± 12.5 (5-52)	/

SD, standard deviation

The genotype and allele frequencies of the GABRG2 rs211037 polymorphism were determined in all participants (Table 2). The distributions of analysed genotype frequencies were within the Hardy–Weinberg equilibrium (p>0.05) in both IGE patient and control groups.

Table 2. Genotype frequencies in patients with idiopathic generalized epilepsy (IGE) patients and controls

GABRG2 rs211037 polymorphism	**No (%) of subjects in the group**	
	IGE patients	**Controls**
Genotype		
CC	72 (75.00)	30 (58.82)
CT	20 (20.83)	17 (33.33)
TT	4 (4.17)	4 (7.84)
Allele		
C	164 (85.42)	77 (75.49)
T	28 (14.58)	25 (24.51)

The homozygous wild CC genotype was overrepresented among the IGE patients when compared against the control group. Genetic analysis reveals a significant association of the

CC genotype using the dominant model (OR=2.100, 95% CI=1.018-4.332; p=0.043) (Table 3). Carriers of CC genotype had approximately a two-fold higher probability for the development of IGE than the carriers of heterozygous CT and homozygous TT genotypes. Similarly, a significant association of C allele with IGE susceptibility was found by the allelic model (OR=1.902, 1.040-3.477; p=0.035) implying that the carriers of C allele had 1.9 folds higher predisposition to IGE when compared to those with a T allele.

Table 3. Genotypes and allele distribution

Genetic model	**Genotype**	**OR (95% CI)**	**p**
Dominant	CC vs. CT + TT	2.100 (1.018 to 4.332)	0.043
Allelic	C vs. T	1.902 (1.040 to 3.477)	0.035

DISCUSSION

This is the first study to evaluate the association of GABRG2 rs211037 polymorphism with the susceptibility to idiopathic generalized epilepsy in the population of patients in the Republic of

North Macedonia. We found that the presence of C allele variant and CC genotype increased by the 2-fold probability of developing IGE in the Macedonian population. To our best knowledge, there is only one prior study evaluating this genetic association in Southeast Europe (15). Interestingly, in the former case-control study from Romania evaluating 114 children with IGE or FS, GABRG2 Asn196Asn TT genotype polymorphism was found to carry a 45 and 8 times higher risk of developing IGE and recurrent FS, respectively (15). A higher frequency of the TT genotype and T allele of the C588 T polymorphism of the GABARG2 gene in patients than controls were also found in the Egyptian cohort of children with IGE (17). In a similar cohort of all-encompassing IGE types in childhood population from Taiwan, the relative risk of IGE in individuals with the GABRG2 rs211037-CC genotype was estimated to be 3.61 times greater compared with those with the GABRG2 rs211037-TT genotype (14) the γ2 subunit of GABA receptor (SNP211037; there was a higher frequency of the TT genotype and T allele of the C588 T polymorphism of the GABARG2 gene in patients than controls. On the contrary, no positive association with IGE was established in a study examining the role of several GABRG2 gene single nucleotide polymorphisms (including rs211037) in two separate cohorts from England and Ireland (18).

The results for distinct subgroups of IGE are also inconsistent. Namely, CC genotype and the C allele were found to be significantly overrepresented in the patients with JME in a study from India (9), while there was a lack of association between rs211037 of the GABRG2 gene and JME in another Indian study (19) as well as Brazilian population (13). In the two studies regarding CAE exclusively, allele and genotype frequencies of the rs211037 of the GABRG2 gene polymorphisms did not differ significantly between CAE patients and healthy controls in the German and Korean population, respectively (20,21). Of note, more than half of the patients in our cohort were diagnosed with JME, while only a few with CAE. However, we did not evaluate subsyndromes separately because the relatively small number of participants in each group is inadequate for statistical analyses.

Apart from IGE, GABRG2 rs211037 (CC genotype/C allele) was found to be a risk factor for febrile seizures, focal seizures, and symptomatic epilepsy (SE) in Asians, particularly in Chinese (12). On the other side, several studies failed to present any evidence for association of GABRG2 rs211037 polymorphism with epilepsy susceptibility in general (10), as well as in different cohorts of temporal lobe epilepsy (TLE): familial TLE preceded by FS in the USA cohort (22), TLE with hippocampal sclerosis in the UK and Ireland patients (18) and mesial TLE in Indian population (23). Further, no association was found between GABRG2 rs211037 polymorphism and susceptibility of Lennox-Gastaut syndrome (19).

The possible explanation for those divergent findings might be that there is population-specific variation implicated in influencing susceptibility to disease. Then, the sample size is a fundamental determinant of the power to identify a causal variant in genetic association studies, and large populations of patients with epilepsy are needed to establish the role of common genetic variants with small effect sizes. The combined results of recent meta-analysis and expression quantitative trait loci analysis, which evaluated the role of GABRG2 in epilepsy, indicated that the GABRG2 C588T polymorphism was associated with IGE risk under dominant and allelic models (24). Further polygenic risk scores that combine the effect sizes of numerous variants into a single score can probably better stratify affected and healthy individuals (25).

A substantial number of antiseizure medications (ASM) target the $GABA_A$ receptor, for the benzodiazepine and barbiturates; it is the primary or only known mechanism of antiseizure action, while for topiramate, felbamate, and stiripentol, $GABA_A$ receptor modulation is one of several possible antiseizure mechanisms (26). Few studies examined the possible association of GABRG2 rs211037 polymorphism and drug-resistance in patients with epilepsy (9,10,17). While in childhood cohort with IGE from Egypt, there was a substantial increase of the T allele among drug-resistant patients compared with those responding to ASM, i.e. children with the C allele were four times more likely to be responsive ASM than non-C-allele- carriers (17) no correlation was found in other studies (9-10). In the Wang

et al. meta-analysis it was found that the protein encoded by the GABRG2 gene interacted with 61 drugs, some of which are currently approved ASM, and others that have not yet been approved for epilepsy treatment but may have antiseizure potential, and therefore, GABRG2 might be a potential therapeutic target for epilepsy (24).

Although only a relatively small number of pathogenic mutations involve genes 'actionable' with current therapeutic tools (27), the therapeutic strategy of the future might involve, among others, enhancement of the wild-type $GABA_A$ receptor channel function (28).

The main limitation of the study is the small number of patients with CAE in our cohort. The reason for underrepresentation is that recruitment process took place at teenage and adult epilepsy clinic (where patients older than 14 years are treated), while CAE is usually resolved by the age of 14 and only an insignificant number of patients continue to have seizures after this age. Since lack of a sufficient number of participants in each group would prevent accurate statistical evaluation and conclusions, we did not break them down into subcategories and perform analysis for the whole group of IGE.

In conclusion, the results from our study have established the association of polymorphism rs211037 of the GABRG2 gene with the susceptibility to idiopathic generalized epilepsy in the Macedonian population.

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TRANSPARENCY DECLARATION

Conflicts of interest: None to declare.

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