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ORIGINAL ARTICLE

Evaluation of GABBR2 gene polymorphisms rs230416 and rs230429 in migraine with aura: A case-control study in a Turkish population

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ABSTRACT

Objectives: This study aimed to investigate the association between two single-nucleotide polymorphisms, rs230416 and rs230429, in the GABBR2 (gamma-aminobutyric acid type B receptor subunit 2) gene and susceptibility to migraine with aura in a Turkish cohort.

Patients and methods: In this case-control study, a total of 100 patients (15 males, 85 females; mean age: 33.68 ± 12.16 years; range, 18 to 55 years) with migraine diagnosed according to the International Classification of Headache Disorders criteria and 100 age- and sex-matched healthy controls (25 males, 75 females; mean age: 33.68 ± 12.16 years; range, 18 to 55 years) were genotyped using the polymerase chain reaction-restriction fragment length polymorphism method between January 2018 and June 2013. Genotype and allele frequencies were compared between groups. The Hardy–Weinberg equilibrium, the chi-square test, and Fisher's exact test were used for statistical analysis ($p < 0.05$). Power analysis and genetic equilibrium testing were also performed.

Results: For rs230416, genotype distributions were 10% GG, 30% AG, and 60% AA in migraineurs, and 5% GG, 25% AG, and 70% AA in controls ($p = 0.15$). Minor allele (G) frequencies were 25% in cases and 17.5% in controls ($p = 0.07$). For rs230429, distributions were 10% TT, 38% CT, and 52% CC in patients, and 8% TT, 34% CT, and 58% CC in controls ($p = 0.42$), with T allele frequencies of 29% and 25%, respectively ($p = 0.45$). All genotype distributions conformed to the Hardy-Weinberg equilibrium.

Conclusion: Although this study did not find a significant association between rs230416 or rs230429 and migraine susceptibility, the findings contribute to the growing literature supporting a minor but biologically relevant role of gamma-aminobutyric acid-mediated pathways in migraine. Future studies with larger, ethnically diverse cohorts and integrative omics approaches are warranted to clarify the functional impact of GABBR2 variants.

Keywords: GABA-B receptor, GABBR2, Migraine with aura, rs230416, rs230429.

Migraine is a prevalent and disabling neurological disorder affecting approximately 12% of the global adult population. Characterized by recurrent attacks of headache often accompanied by photophobia, phonophobia, nausea, and sometimes focal neurological auras, migraine disproportionately affects females, with a female-to-male ratio of nearly 3:1, suggesting a complex interplay of hormonal and genetic factors in its pathophysiology.[1-3]

Based on clinical and pathophysiological criteria, migraine is classified into two major subtypes: migraine with aura (MA) and migraine without aura (MO). Increasing evidence suggests that MA may have a stronger genetic predisposition compared to MO, making it a more suitable phenotype for genetic association studies.[4,5] Therefore, to enhance phenotypic homogeneity and minimize diagnostic ambiguity, the present study focused exclusively on individuals with MA.

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The underlying mechanisms of migraine are multifactorial, involving genetic susceptibility, neurovascular dysfunction, cortical spreading depolarization, neuroinflammation, and imbalance between excitatory and inhibitory neurotransmitter systems within the central nervous system. Among these, the gamma-aminobutyric acid (GABA)-mediated (GABAergic) system has garnered significant attention due to its role in modulating cortical excitability and pain thresholds. Gamma-aminobutyric acid is the primary inhibitory neurotransmitter in the brain, and impaired GABAergic function has been implicated in increased neuronal excitability in migraineurs.[6,7]

Gamma-aminobutyric acid type B receptors (GABA-B) are metabotropic G-protein-coupled receptors that mediate slow and prolonged inhibitory synaptic transmission by suppressing presynaptic neurotransmitter release. The GABBR2 gene, which encodes the GABA-B subunit 2, is essential for receptor assembly and signaling. Variants within this gene have been previously associated with several neuropsychiatric conditions and are now being investigated for their potential role in migraine susceptibility.[8]

Among these, rs230416 and rs230429 are common single-nucleotide polymorphisms (SNPs) within the GABBR2 gene that have drawn attention due to their potential functional relevance. Specifically, rs230416 is located within an intronic region, whereas rs230429 is a synonymous coding variant situated within an exon that, despite not altering the amino acid sequence, may still influence mRNA stability, splicing efficiency, or translational dynamics. Although these SNPs have not been widely studied in migraine populations, preliminary data from functional annotation studies suggest a potential role in GABAergic neurotransmission, possibly through modulation of receptor expression or function. Given the complex genetic architecture of migraine and potential population-specific effects, further investigation in diverse ethnic cohorts is warranted to clarify their contribution to disease susceptibility.[9,10]

In this context, we aimed to investigate the association between two GABBR2 polymorphisms (rs230416 and rs230429) and MA in a clinically homogeneous Turkish cohort. Genotyping was performed using the polymerase chain reaction PCR-restriction fragment length polymorphism (PCR-RFLP) method, which provides a cost-effective and reliable approach for targeted SNP analysis. However, we acknowledge that RFLP-based genotyping is inherently limited to predefined variants and may not capture rare genetic variants or complex haplotype structures within the GABBR2 gene. Accordingly, our findings should be interpreted within the context of the selected SNPs, and future studies employing high-throughput sequencing or haplotype-based analyses may offer a more comprehensive assessment of GABAergic genetic contributions to migraine susceptibility.

PATIENTS AND METHODS

In this case-control study, a total of 100 patients (15 males, 85 females; mean age: 33.68 ± 12.16 years; range, 18 to 55 years) diagnosed with MA according to the International Classification of Headache Disorders[11] criteria and 100 age- and sex-matched healthy controls (25 males, 75 females; mean age: 33.68 ± 12.16 years; range, 18 to 55 years) were recruited from healthcare institutions across Türkiye between January 2018 and June 2013. The control group consisted of neurologically healthy individuals with no personal or family history of migraine or other chronic neurological conditions. Demographic characteristics such as age, sex, and lifestyle factors (e.g., smoking, caffeine intake, and sleep habits) were matched between the two groups to minimize potential confounding effects.

A written informed consent was obtained from each patient. The study protocol was approved by the Atatürk University Faculty of Medicine Ethics Committee (Date 16.05.2012, Decision no: 211). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Isolation of DNA

Peripheral venous blood samples were collected into EDTA-containing tubes from all participants. Genomic DNA was extracted using the standard salting-out method described by Miller et al.[12] The concentration and purity of the isolated DNA samples were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and only samples with adequate purity (A260/A280 ratio between 1.8 and 2.0) were included for further analysis.

Polymerase chain reaction amplification

Primers specific to the regions flanking the target SNPs were designed using Primer3 software. Polymerase chain reaction was carried out in a total volume of 25 µL containing 100 ng of

genomic DNA, 0.2 mM of each dNTP, 0.5 µM of each forward and reverse primer, 2 mM magnesium chloride, and 1 U of HotStart Taq DNA polymerase (Thermo Fisher Scientific or equivalent).

The thermal cycling protocol included an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at primer-specific temperatures for 30 sec, and extension at 72°C for 45 sec. A final extension was performed at 72°C for 7 min. Polymerase chain reaction products were separated by electrophoresis on 2% agarose gels stained with ethidium bromide and visualized under ultraviolet light.

Restriction enzyme digestion and genotyping

Polymerase chain reaction products were subjected to restriction enzyme digestion using EcoRI and HindIII, selected based on their ability to recognize and cleave at the SNP-altered restriction sites. The efficiency and specificity of the enzymes were validated prior to the experiment, and all digestions were performed under optimal conditions recommended by the manufacturer.

Following enzymatic digestion, the resulting DNA fragments were separated by electrophoresis on 2% agarose gels stained with ethidium bromide and visualized under ultraviolet illumination. Genotypes were determined based on the observed banding patterns: homozygous wild-type, heterozygous, or homozygous variant, depending on the presence or absence of the expected restriction fragments.

Statistical analysis

Genotype and allele frequencies were calculated using IBM SPSS version 25.0 (IBM Corp., Armonk, NY, USA). The Hardy-Weinberg equilibrium was separately assessed in both case and control groups using the chi-square test. Differences in genotype and allele distributions between patients with migraine and healthy controls were evaluated using the chi-square test or Fisher's exact test, as appropriate, and p-values were derived from these tests. A p-value <0.05 was considered statistically significant. In addition, power analysis and genetic equilibrium testing were performed to ensure the reliability and validity of the study findings.

RESULTS

The observed genotype and allele frequencies for both the patient and control groups are summarized in Table 1. For the rs230416 polymorphism, the genotype frequencies in the migraine group were 10% homozygous mutant (GG), 30% heterozygous (AG), and 60% homozygous wild-type (AA). In the control group, the respective frequencies were 5% GG, 25% AG, and 70% AA. Genotype distributions in both groups were consistent with Hardy-Weinberg equilibrium (p > 0.05). There was no statistically significant difference in genotype distribution between the patients with migraine and healthy controls (p = 0.15). The frequency of the minor allele (G) was found to be 25% in the migraine group and 17.5% in the control group, a difference that did not reach statistical significance (p = 0.07).

TABLE 1
Genotype and allele distributions of GABBR2 polymorphisms in migraine and control groups

		Migraine group (n = 100)		Control group (n = 100)		
SNP (GABBR2)	Genotype/allele	n	%	n	%	p
	AA	60	60	70	70	0.15
	AG	30	30	25	25	
rs230416	GG	10	10	5	5	
	Allele A	150	75	165	82.5	0.07
	Allele G	50	25	35	17.5	
	CC	52	52	58	58	0.42
	CT	38	38	34	34	
rs230429	TT	10	10	8	8	
	Allele C	142	71	150	75	0.45
	Allele T	58	29	50	25	

SNP, single nucleotide polymorphism.

Similarly, for the rs230429 polymorphism, genotype frequencies in the migraine group were 10% homozygous mutant (TT), 38% heterozygous (CT), and 52% homozygous wild-type (CC), while the corresponding frequencies in the control group were 8% TT, 34% CT, and 58% CC. No statistically significant differences were observed in genotype distribution between the two groups (p = 0.42). The minor allele (T) frequency was 29% in the migraine group and 25% in the control group, with no statistically significant difference (p = 0.45). Genotype distributions for both groups were consistent with the Hardy-Weinberg equilibrium ($p > 0.05$).

DISCUSSION

In this study, we examined the potential association between two GABBR2 gene polymorphisms (rs230416 and rs230429) and MA using PCR-RFLP-based genotyping. No statistically significant differences were found between patients with migraine and controls. This may reflect the modest effect sizes of individual GABAergic variants, as suggested by previous studies, as well as the limited sample size or population-specific genetic variability. While PCR-RFLP was a suitable method for preliminary screening, further studies with larger cohorts and high-throughput genotyping are warranted to elucidate the role of GABBR2 in migraine pathogenesis.

Large-scale genome-wide association studies have identified 123 genetic loci associated with migraine susceptibility, highlighting the disorder's polygenic architecture, and supporting a prominent role for neurovascular and neuronal mechanisms in its pathophysiology.[13] While genes encoding GABA receptors have not emerged as top hits in these genome-wide association studies, the GABAergic system remains a biologically plausible candidate due to its critical role in regulating cortical excitability and synaptic inhibition both key features in migraine pathogenesis. Prior studies suggest that genetic variants influencing GABA signaling may confer modest, population-specific risk, even if not captured in large-scale meta-analyses. Therefore, targeted studies focusing on GABA receptor gene variants, such as the present one, remain valuable to complement genome-wide findings and explore potentially overlooked mechanisms.[10]

Our findings are in line with previous studies suggesting that SNPs in GABA receptor genes may not individually confer a high risk for migraine but rather contribute modestly within a broader polygenic and epigenetic framework. It is plausible that these variants interact with other susceptibility loci or regulatory mechanisms, such as microRNAs or environmental triggers, to influence disease expression. The lack of significant association in our cohort could be attributed to limited statistical power given the sample size, as well as the potentially low penetrance of the examined SNPs. Prior research has emphasized that many migraine-associated genetic variants exhibit small effect sizes and may require large, well-characterized, and ethnically diverse populations to yield reproducible associations.[14]

Several studies support the involvement of GABAergic dysfunction in migraine. A proton magnetic resonance spectroscopy study by Wu et al.[15] reported significantly reduced GABA levels in the anterior cingulate gyrus and medial prefrontal cortex of patients with migraine compared to controls. Peek et al.[16] demonstrated that increased cortical GABA+ levels were associated with reduced migraine attack frequency and intensity. Similarly, expression studies have shown differential regulation of GABA receptor subunits in migraineurs.

The GABBR2 gene, encoding the GABA-B subunit 2, is a G-protein-coupled receptor component that mediates slow, prolonged inhibitory signaling in the central nervous system. Functional disruptions in this receptor could alter pain modulation and neuronal excitability thresholds. Interestingly, a recent preclinical study by Zhang et al.[17] showed that treatment with paeonol and glycyrrhizic acid upregulated GABBR2 expression and attenuated migraine-like behaviors in rats by enhancing GABAergic synaptic signaling.

Moreover, epigenetic mechanisms have also been implicated. For instance, miR-34a, a microRNA found to be elevated in the serum of patients with migraine, has been predicted to target GABBR2 messenger RNA, potentially downregulating its expression and thereby weakening inhibitory GABA-B signaling, which highlights the relevance of gene-environment interactions and epigenetic regulation in migraine.[18]

Although the current study focused on two SNPs, it provides a useful model for evaluating gene-disease associations using PCR-RFLP, a cost-effective and educational genotyping approach. The enzymes used were selected based on their ability to discriminate between alleles, and digestion

conditions were optimized for specificity and reproducibility.[19]

However, several limitations must be acknowledged. First, the relatively small sample size may limit statistical power. Second, the analysis was restricted to two SNPs, which may not capture the full genetic variation within GABBR2. Third, PCR-RFLP, while suitable for low-throughput genotyping, lacks the scalability of next-generation sequencing for broader variant discovery.

In conclusion, while we did not observe statistically significant differences in genotype or allele frequencies of rs230416 and rs230429 between patients with migraine and controls, the broader body of evidence continues to support GABAergic signaling as a promising pathway in migraine pathophysiology. Future investigations would benefit from larger, multi-ethnic cohorts and comprehensive genotyping strategies spanning multiple GABA receptor gene families. In addition, integrating multiomics approaches, including transcriptomic, proteomic, and epigenetic, analyses may help clarify the functional relevance of GABBR2 variants beyond single-SNP associations. Combining genetic data with biochemical markers related to inhibitory neurotransmission, such as GABA levels, glutamate-GABA balance, or inflammatory mediators, may further enhance mechanistic understanding. Moreover, exploring interactions between GABAergic pathways and other neurotransmitter systems, including glutamatergic, serotonergic, and dopaminergic networks, could provide a more holistic view of migraine susceptibility. Finally, pharmacogenetic studies evaluating individual responses to GABA receptor-modulating agents, such as baclofen or GABA A receptor agonists, may offer clinically relevant insights toward personalized therapeutic strategies in migraine management.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: E.Ö.: Idea/Concept, design, data collection, genetic analysis, writing the article; A.T.: Data collection, statistical analysis, literature review; G.Ö.: Study supervision, clinical evaluation of patients, critical review, writing the article.

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