Article

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Multi-Drug Resistance 1 Gene C3435T Polymorphism in Migraine Patients: A Case-Control Study

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Background: Migraine is a complex neurovascular disorder with a substantial genetic component. The multidrug resistance 1 (*MDR1/ABCB1*) gene encodes P-glycoprotein, a key efflux transporter at the blood–brain barrier that regulates the distribution of neuroactive compounds. The functional C3435T polymorphism (rs1045642) has been suggested to influence transporter activity; however, its role in migraine susceptibility remains inconclusive.

Methods: This case–control study included 100 patients with migraine diagnosed according to the International Classification of Headache Disorders, 3rd edition (ICHD-3) and 100 age- and sex-matched healthy controls. Genotyping of the *MDR1* C3435T polymorphism was performed using the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method. Genotype and allele distributions were analyzed under different genetic models. Multivariate logistic regression analysis was conducted to adjust for potential confounders, including age, sex, body mass index, and biochemical parameters. Bonferroni and false discovery rate (FDR) corrections were applied to account for multiple comparisons.

Results: Genotype distributions were consistent with the Hardy–Weinberg equilibrium in both groups ($p > 0.05$). The overall genotype distribution did not differ significantly between migraine patients and controls ($\chi^2 = 4.54$, $p = 0.103$). However, the TT genotype was significantly more frequent in the migraine group (40.0% vs. 30.0%; odds ratio (OR) = 2.38, 95% confidence interval (CI): 1.06–5.34, $p = 0.035$). The dominant model (CT+TT vs. CC) showed borderline significance ($p = 0.052$). The T allele frequency was significantly higher in patients compared to controls (63.0% vs. 52.5%; OR = 1.54, 95% CI: 1.03–2.30, $\chi^2 = 4.519$, $p = 0.034$). However, these associations did not remain statistically significant after Bonferroni and FDR corrections. Multivariate logistic regression analysis yielded comparable results, indicating that the observed associations were not independent of potential confounders.

Conclusion: The *MDR1* C3435T polymorphism may be modestly associated with migraine susceptibility in a genotype-specific manner; however, the lack of significance after correction for multiple testing suggests that this variant is unlikely to represent a strong or independent genetic predictor. Further large-scale, multi-center studies incorporating haplotype analyses and environmental factors are warranted.

Keywords: migraine; *MDR1*; *ABCB1*; C3435T polymorphism; P-glycoprotein; genetic association; case-control study; blood–brain barrier

Introduction

Migraine is a highly prevalent and disabling neurological disorder characterized by recurrent episodes of moderate-to-severe headache, often accompanied by nausea, photophobia, and phonophobia. It affects approximately 15% of the global population and represents a major cause of reduced quality of life and socioeconomic burden worldwide [1–3]. The pathophysiology of migraine is complex and involves interactions between neuronal hyperexcitability, vascular dysregulation, and neuroinflammatory processes [2,3]. Increasing evidence from family-based and twin studies suggests a substantial genetic contribution, with heritability estimates ranging from 40% to 60% [4].

Recent advances in human genetics, including genome-wide association studies (GWAS), have identified multiple susceptibility loci for migraine, implicating pathways related to ion channels, neurotransmitter systems, and vascular regulation [5,6]. In addition to these well-established mechanisms, emerging evidence highlights the potential role of the blood–brain barrier (BBB) and its transport systems in migraine pathophysiology [7]. The BBB is a highly selective interface that regulates the exchange of substances between the systemic circulation and the central nervous system, and its dysfunction may alter the homeostasis of neuroactive compounds involved in migraine [7,8].

The multidrug resistance 1 (*MDR1*, also known as *ABCB1*) gene encodes P-glycoprotein, an ATP-dependent

efflux transporter located on the luminal surface of BBB endothelial cells. P-glycoprotein plays a critical role in limiting the penetration of xenobiotics and endogenous neuroactive substances into the brain, thereby contributing to neuroprotection and pharmacokinetic regulation [9,10]. Alterations in P-glycoprotein expression or function may influence both susceptibility to neurological disorders and variability in drug response, particularly in conditions such as epilepsy, depression, and migraine [11–13].

Among the known genetic variants of *MDR1*, the C3435T polymorphism (rs1045642) has been extensively studied due to its potential functional significance. Although it is a synonymous variant, it has been reported to affect mRNA stability, protein folding, and substrate specificity, ultimately influencing P-glycoprotein expression and activity [14,15]. Individuals carrying the TT genotype have been associated with reduced transporter function, which may lead to increased central nervous system exposure to neuroactive compounds such as serotonin and glutamate—key mediators implicated in migraine pathophysiology [16,17].

Despite this plausible biological link, previous studies investigating the association between *MDR1* polymorphisms and migraine have yielded inconsistent findings. Most of the available research has focused on pharmacogenetic outcomes, particularly treatment response to antimigraine therapies, rather than on disease susceptibility itself [18,19]. Furthermore, the number of studies evaluating this association in specific populations, including the Turkish population, remains limited. Given the potential influence of genetic background and population heterogeneity on association studies, further investigation in well-characterized cohorts is warranted.

Therefore, the present study aimed to investigate the association between the *MDR1* C3435T polymorphism and migraine susceptibility in a Turkish population using a case–control design. In addition, we sought to improve methodological rigor by incorporating multivariate logistic regression analyses to control for potential confounding factors and by applying multiple comparison corrections to ensure the robustness of the findings.

Methods

Study Design and Population

This case–control study included a total of 200 participants, comprising 100 patients diagnosed with migraine and 100 healthy control subjects. Patients were recruited from the Neurology Outpatient Clinic of Çanakkale Onsekiz Mart University Hospital, Çanakkale, Türkiye, between May 2023 and December 2025. Migraine diagnosis was established according to the International Classification of Headache Disorders, 3rd edition (ICHD-3) criteria [20]. All patients were evaluated by a neurologist through a detailed clinical assessment, including headache characteristics, frequency, duration, and associated symptoms.

Participants were recruited from the same geographic region and healthcare setting to minimize potential population stratification bias. The study protocol was approved by the Clinical Research Ethics Committee of Çanakkale Onsekiz Mart University (Decision No: 2023/07-02; Date: May 03, 2023; Application No: 2023-46), and all procedures were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrollment.

Inclusion Criteria

Participants were eligible for inclusion if they met the following criteria:

Age between 18 and 65 years;

For the patient group: confirmed diagnosis of migraine (with or without aura) according to ICHD-3 criteria [20];

For the control group: absence of any history of migraine or other primary headache disorders;

No history of neurological or chronic systemic diseases;

No regular use of medications affecting central nervous system function, oxidative stress, or drug metabolism;

Willingness to participate and provision of written informed consent.

Exclusion Criteria

Participants were excluded if they had:

Chronic systemic diseases (e.g., diabetes mellitus, cardiovascular disease);

Psychiatric disorders;

Pregnancy or lactation;

Chronic use of medications influencing metabolic or detoxification pathways;

History of neurological disorders other than migraine.

Healthy controls were selected from volunteers without any history of migraine, neurological disease, or chronic illness to ensure comparability between groups.

Sample Size and Power Analysis

A priori power analysis indicated that a minimum of 90 participants per group would be required to detect a 20% difference in genotype frequencies with 80% statistical power at a significance level of $\alpha = 0.05$. Therefore, 100 participants were included in each group to increase statistical robustness and account for potential data loss.

DNA Extraction and Genotyping of MDR1 C3435T Polymorphism

Peripheral venous blood samples (5 mL) were collected in EDTA-containing tubes. Genomic DNA was extracted using the standard phenol–chloroform method. DNA concentration and purity were assessed spectrophotometrically using A260/A280 ratios, and samples were stored at –20 °C until analysis.

Genotyping of the *MDR1* C3435T polymorphism (rs1045642) was performed using the polymerase chain

reaction–restriction fragment length polymorphism (PCR-RFLP) method, as previously described [14]. The polymorphism was selected due to its well-documented functional relevance and frequent investigation in pharmacogenetic and neurological studies [9,21].

PCR amplification was carried out in a total reaction volume of 50 µL containing approximately 100 ng genomic DNA, 10× PCR buffer, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 0.4 µM of forward and reverse primers, 1 U of Taq DNA polymerase, and nuclease-free water. The primer sequences used were:

Forward: 5′-TTGATGGCAAAGAAATAAAGC-3′;

Reverse: 5′-CTTACATTAGGCAGTGACTCG-3′.

PCR conditions included an initial denaturation step followed by amplification cycles (details available upon request). The amplified products were digested overnight at 37 °C using the restriction enzyme MboI.

The resulting fragments were separated by electrophoresis on a 3% agarose gel stained with ethidium bromide and visualized under UV illumination. Genotypes were identified based on fragment sizes:

TT genotype: 207 bp (undigested);

CT genotype: 207, 145, and 62 bp fragments;

CC genotype: 145 and 62 bp fragments.

To ensure genotyping accuracy, 10% of the samples were randomly selected and re-analyzed, yielding 100% concordance.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were presented as frequencies and percentages.

Data Distribution and Group Comparisons

The normality of continuous variables was assessed using the Shapiro–Wilk test. Variables showing normal distribution were expressed as mean ± standard deviation (SD) and compared using the independent samples t-test. Triglyceride levels, which did not conform to a normal distribution, were expressed as median (interquartile range, IQR) and analyzed using the Mann–Whitney U test. Categorical variables were analyzed using the chi-square (χ^2) test.

Genetic Association Analysis

Genotype and allele frequencies were calculated for both groups, and the Hardy–Weinberg equilibrium (HWE) was assessed using the chi-square test. Genetic associations were evaluated under different inheritance models:

- Codominant model (CC vs. CT vs. TT);
- Dominant model (CC vs. CT+TT);
- Recessive model (TT vs. CC+CT).

Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate effect sizes.

Multivariate Analysis

To control for potential confounding factors, multivariate logistic regression analysis was performed with migraine status as the dependent variable. Independent variables included:

- *MDR1* C3435T genotype and allele status;
- Age;
- Gender;
- Body mass index (BMI);
- Biochemical parameters (triglycerides, total cholesterol, LDL-C, HDL-C, vitamin D, and vitamin B12 levels).

Adjusted odds ratios (aORs) with 95% confidence intervals were reported.

Multiple Comparison Correction

To reduce the risk of type I error due to multiple comparisons, statistical significance was further evaluated using:

- Bonferroni correction;
- False discovery rate (FDR) correction (Benjamini–Hochberg method).

A corrected p-value < 0.05 was considered statistically significant.

Results

Baseline Characteristics of the Study Population

The baseline characteristics of the study groups are presented in Table 1.

There were no statistically significant differences between migraine patients and controls in terms of age, BMI, or biochemical parameters (all $p > 0.05$). These findings indicate that the two groups were comparable, minimizing potential confounding effects.

The mean age of migraine patients was 35.10 ± 9.43 years, compared to 33.61 ± 10.24 years in the control group, with no statistically significant difference ($t = 1.07$, $p = 0.286$). Similarly, body mass index (BMI) did not differ significantly between groups (26.93 ± 5.53 vs. 29.24 ± 11.63 kg/m^2; $t = –1.80$, $p = 0.074$).

Triglyceride levels were presented as median (IQR) because they did not follow a normal distribution. No statistically significant difference was observed between migraine patients and controls [114 (73–178) vs. 123 (87–175) mg/dL; z = –0.50, $p = 0.620$].

No significant differences were observed in other biochemical parameters, including total cholesterol ($t = –0.53$, $p = 0.599$), LDL-C ($t = –1.14$, $p = 0.254$), HDL-C ($t = –1.37$, $p = 0.171$), vitamin D ($t = 1.50$, $p = 0.136$), and vitamin B12 ($t = 1.79$, $p = 0.075$), as shown in Table 1.

These findings indicate that the migraine and control groups were comparable with respect to baseline demo-

Table 1. Socio-demographic and clinical characteristics of the study groups.

Variables	Cases (n = 100)	Control (n = 100)	*t*/z value	*p* value
	mean ± SD	mean ± SD		
Age (years)	35.10 ± 9.43	33.61 ± 10.24	1.07	0.286
BMI (kg/m^2)	26.93 ± 5.53	29.24 ± 11.63	–1.80	0.074
TG (mg/dL)	114 (73–178)	123 (87–175)	–0.50	0.620
TC (mg/dL)	187.04 ± 39.54	190.16 ± 44.04	–0.53	0.599
LDL-C (mg/dL)	105.82 ± 34.15	111.14 ± 31.60	–1.14	0.254
HDL-C (mg/dL)	61.06 ± 10.92	64.18 ± 19.83	–1.37	0.171
Vitamin D (ng/mL)	19.95 ± 8.34	18.24 ± 7.77	1.50	0.136
Vitamin B12 (pg/mL)	327.82 ± 59.76	300.17 ± 142.49	1.79	0.075

Data are presented as mean ± standard deviation (SD), except for triglycerides, which are presented as median (interquartile range, IQR). BMI, Body Mass Index; TG, Triglycerides; TC, Total Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; HDL-C, High-Density Lipoprotein Cholesterol; SD, Standard Deviation.

Table 2. Clinical characteristics of migraine patients.

Clinical features	Migraine patients (n = 100)
Without aura	64 (64.0%)
With aura	36 (36.0%)
Photophobia	95 (95.0%)
Phonophobia	93 (93.0%)
Nausea	77 (77.0%)
Vomiting	37 (37.0%)

graphic and metabolic characteristics, minimizing potential confounding effects.

Clinical Characteristics of Migraine Patients

The clinical characteristics of migraine patients are summarized in Table 2.

Among the 100 patients, 64% were classified as migraine without aura, while 36% had migraine with aura. The most frequently reported associated symptoms were photophobia (95%), phonophobia (93%), and nausea (77%), whereas vomiting was reported in 37% of patients. These findings confirm the typical clinical profile of migraine within the study population.

Genotype and Allele Distributions

The genotype and allele distributions of the *MDR1* C3435T polymorphism are presented in Table 3.1, Table 3.2 and Table 3.3. Genotype distributions in both migraine patients and controls were consistent with Hardy–Weinberg equilibrium (HWE) ($p > 0.05$), supporting the reliability of the genotyping procedure.

The overall genotype distribution did not differ significantly between the two groups ($\chi^2 = 4.54$, $p = 0.103$), as shown in Table 3.1 However, in the codominant model, the TT genotype was significantly more frequent in migraine patients compared to controls (40.0% vs. 30.0%; OR = 2.38, 95% CI: 1.06–5.34, $p = 0.035$).

In the dominant model (CT+TT vs. CC), a borderline association was observed (OR = 2.05, 95% CI: 0.99–4.22, $p = 0.052$), whereas no statistically significant difference was detected in the recessive model (TT vs. CC+CT) (OR = 1.56, 95% CI: 0.87–2.79, $p = 0.154$) (Table 3.2).

At the allele level, the frequency of the T allele was significantly higher in migraine patients compared to controls (63.0% vs. 52.5%; $\chi^2 = 4.519$, $p = 0.034$; OR = 1.54, 95% CI: 1.03–2.30) (Table 3.3).

Multivariate Logistic Regression Analysis

To evaluate whether the observed associations were independent of potential confounding factors, multivariate logistic regression analysis was performed, adjusting for age, gender, BMI, and biochemical parameters (triglycerides, total cholesterol, LDL-C, HDL-C, vitamin D, and vitamin B12 levels).

As summarized in Table 3.1,3.2,3.3, the adjusted analysis yielded results comparable to the univariate findings. The TT genotype remained associated with an increased odds of migraine; however, the strength of the association did not increase, and statistical significance was not consistently robust after adjustment. These findings indicate that the observed association is not fully explained by measured confounders but also does not represent a strong independent effect.

Multiple Comparison Correction

To address multiple testing, Bonferroni correction and false discovery rate (FDR) adjustment (Benjamini–Hochberg method) were applied to the genotype and allele analyses presented in Table 3.1,3.2,3.3. Based on the final revised results, the TT genotype ($p = 0.035$) and the T allele ($p = 0.034$; $\chi^2 = 4.519$) showed nominal statistical significance. However, these associations did not remain statistically significant after Bonferroni and FDR corrections (adjusted $p > 0.05$). Therefore, the observed associations

Table 3.1. Genotype distribution of the *MDR1* C3435T polymorphism (codominant model).

Genotype	Cases (n = 100, %)	Controls (n = 100, %)	OR (95% CI)	χ^2	p_1 (χ^2)	p_2 (OR)
CC	14 (14.0%)	25 (25.0%)	1.00 (Reference)	4.54	0.103	-
CT	46 (46.0%)	45 (45.0%)	1.83 (0.84–3.95)			0.127
TT	40 (40.0%)	30 (30.0%)	2.38 (1.06–5.34)			0.035

p_1: overall chi-square comparison; p_2: OR significance.

Table 3.2. Genetic model analysis of the *MDR1* C3435T polymorphism.

Model	Comparison	Cases (%)	Controls (%)	OR (95% CI)	*p*-value
Dominant	CT+TT vs. CC	86.0% vs. 14.0%	75.0% vs. 25.0%	2.05 (0.99–4.22)	0.052
Recessive	TT vs. CC+CT	40.0% vs. 60.0%	30.0% vs. 70.0%	1.56 (0.87–2.79)	0.154

Table 3.3. Allele distribution of the *MDR1* C3435T polymorphism.

Allele	Cases (n = 200, %)	Controls (n = 200, %)	OR (95% CI)	χ^2	*p*-value
C	74 (37.0%)	95 (47.5%)	(1.0) Reference		
T	126 (63.0%)	105 (52.5%)	1.54 (1.03–2.30)	4.519	0.034

Notes:

• Total number of individuals in each group is n = 100.

• Allele counts are based on 2n (total alleles = 200 per group).

• Combined genotype groups (CT+TT and CC+CT) are used only for genetic model analysis and do not represent additional independent samples.

should be interpreted cautiously, as they may be influenced by multiple comparisons rather than representing robust independent effects.

Overall Interpretation of Results

Taken together, the results presented in Tables 1,2,3.1,3.2,3.3 demonstrate that while the overall genotype distribution of the *MDR1* C3435T polymorphism does not differ significantly between migraine patients and controls, the increased frequency of the TT genotype and T allele suggests a modest, genotype-specific association. However, the lack of consistent significance across genetic models, the absence of a strong independent effect in multivariate analysis, and the loss of significance after multiple testing correction indicate that this polymorphism is unlikely to be a major determinant of migraine susceptibility.

Discussion

In the present case–control study, we investigated the association between the *MDR1* (*ABCB1*) C3435T polymorphism and migraine susceptibility in a Turkish population. Although no statistically significant difference was observed in the overall genotype distribution between migraine patients and controls, a higher frequency of the TT genotype and T allele was identified in the migraine group. These findings suggest a modest and genotype-specific association rather than a strong or consistent genetic effect. Importantly, after adjustment for potential confounders and correction for multiple comparisons, the observed associations did not remain statistically significant, indicating that the results should be interpreted with caution.

From a biological perspective, the *MDR1* gene encodes P-glycoprotein, an ATP-dependent efflux transporter that plays a critical role in maintaining blood–brain barrier (BBB) integrity by regulating the transport of endogenous and exogenous compounds [8,16]. Alterations in P-glycoprotein function may influence the central nervous system exposure to neuroactive substances, including serotonin, dopamine, and glutamate, which are key mediators in migraine pathophysiology [14,17]. Therefore, genetic variation in *MDR1* may theoretically contribute to migraine susceptibility by modifying BBB permeability and neurotransmitter homeostasis.

The C3435T polymorphism (rs1045642), although synonymous, has been reported to affect mRNA stability, protein folding, and substrate specificity of P-glycoprotein [18,21]. Individuals carrying the TT genotype have been associated with reduced transporter expression and efflux capacity, which could result in increased accumulation of neuroactive compounds in the brain [21,22]. This mechanism provides a plausible explanation for the higher frequency of the TT genotype observed in migraine patients in our study. However, it should be emphasized that the functional consequences of this polymorphism remain inconsistent across studies, and its direct role in migraine pathogenesis has not been conclusively established. Despite this biological plausibility, the functional consequences of the C3435T polymorphism remain inconsistent across studies. Furthermore, although nominal statistical significance was observed for the TT genotype and T allele (p = 0.035 and

$p = 0.034$, respectively), these associations did not remain significant after Bonferroni and FDR corrections. This suggests that the observed findings may be influenced by multiple testing and should therefore be interpreted cautiously.

Previous studies investigating *MDR1* polymorphisms in migraine have primarily focused on pharmacogenetic aspects, particularly treatment response to antimigraine drugs, rather than disease susceptibility [12,19,23]. Some studies have suggested that altered P-glycoprotein activity may influence the efficacy of medications such as triptans and antidepressants used in migraine prophylaxis [24]. In contrast, evidence supporting a direct association between *MDR1* polymorphisms and migraine risk remains limited and inconclusive. Our findings are partially consistent with earlier reports indicating a potential, but modest, role of P-glycoprotein in neurological disorders and headache-related conditions [24,25].

Importantly, although nominal statistical significance was observed for the TT genotype and T allele in the present study, these associations did not withstand Bonferroni and false discovery rate (FDR) corrections. This suggests that the findings may be influenced by multiple testing and highlights the importance of applying appropriate statistical adjustments in genetic association studies. Similar discrepancies between uncorrected and corrected results have been reported in previous studies investigating single-nucleotide polymorphisms with modest effect sizes [26]. Therefore, the observed associations in this study should be considered preliminary.

Another key aspect of this study is the use of multivariate logistic regression analysis to control for potential confounding factors, including age, gender, BMI, and biochemical parameters. The adjusted analyses yielded results comparable to the univariate findings, indicating that the observed association is not fully explained by these variables. However, the lack of a stronger or independent effect suggests that the contribution of the *MDR1* C3435T polymorphism to migraine susceptibility is likely limited.

Despite matching the study groups for age and gender and adjusting for several clinical variables, residual confounding cannot be completely excluded. Lifestyle-related factors such as smoking, alcohol consumption, sleep quality, and stress levels—known to influence migraine susceptibility—were not systematically assessed. These unmeasured variables may have affected the observed associations and represent an important limitation.

Population stratification is another potential concern in genetic association studies. Although all participants were recruited from the same geographic region to minimize heterogeneity, subtle genetic differences may still exist within the population. This limitation should be considered when interpreting the findings, and future studies should aim to include larger, ethnically homogeneous cohorts or apply genomic control methods.

The relatively small sample size (100 cases and 100 controls) represents another limitation of the present study. Although a priori power analysis indicated adequate power to detect moderate genetic effects, smaller effect sizes may not have been captured. This limitation may contribute to borderline statistical findings and increase the risk of both type I and type II errors. Larger, multicenter studies are therefore required to validate these findings.

Another limitation is the evaluation of only a single polymorphic site within the *MDR1* gene. Given that *MDR1* is highly polymorphic, other variants such as G2677T/A and C1236T—particularly when analyzed as haplotypes—may provide a more comprehensive understanding of the genetic contribution of P-glycoprotein to migraine [19,26]. Future studies incorporating haplotype-based analyses and gene–gene interactions are warranted.

Despite these limitations, the present study has several strengths, including a well-defined study population, standardized diagnostic criteria, and rigorous statistical analysis with multiple testing correction and independent data verification. Furthermore, the integration of clinical and biochemical parameters into multivariate models enhances the reliability of the findings.

In addition, recent advances in migraine research emphasize the importance of BBB integrity and transporter function in disease pathophysiology [7]. Emerging evidence suggests that altered BBB permeability and transporter dysfunction may influence not only disease susceptibility but also treatment response. Therefore, further investigation of *MDR1* polymorphisms in combination with functional and pharmacogenomic studies may provide valuable insights into personalized approaches for migraine management.

Conclusion

In conclusion, this case–control study investigated the potential association between the *MDR1* (*ABCB1*) C3435T polymorphism and migraine susceptibility in a Turkish population. Although the overall genotype distribution did not differ significantly between migraine patients and healthy controls, a higher frequency of the TT genotype and T allele was observed in the migraine group, suggesting a possible genotype-specific effect. However, this association was not consistently supported across different genetic models and did not remain statistically significant after adjustment for multiple comparisons, indicating that the observed findings are modest and should be interpreted with caution. Furthermore, multivariate logistic regression analysis demonstrated that the *MDR1* C3435T polymorphism does not constitute a strong or independent predictor of migraine risk when potential confounding factors are taken into account.

Taken together, these results suggest that the contribution of the *MDR1* gene to migraine susceptibility is limited and likely reflects a minor component within a com-

plex, multifactorial disease process involving multiple genetic, environmental, and neurobiological factors. Therefore, this polymorphism alone cannot be considered a reliable biomarker for migraine risk. Future research should focus on larger, well-powered, and ethnically diverse populations, incorporating multiple genetic variants, haplotype-based analyses, and gene–environment interactions to better elucidate the role of drug transporters and blood–brain barrier dynamics in migraine pathophysiology. Such integrative approaches may ultimately contribute to the development of more precise and individualized therapeutic strategies.

Abbreviations

PCR-RFLP, Polymerase chain reaction–restriction fragment length polymorphism; BMI, Body mass index; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; TC, Total cholesterol; TG, Triglycerides; MDR1, Multidrug Resistance 1; OR, Odds ratio; CI, Confidence interval; BBB, Blood–brain barrier; HWE, Hardy–Weinberg equilibrium; FDR, False discovery rate; ICHD-3, International Classification of Headache Disorders, 3rd edition.

Availability of Data and Materials

The datasets generated and/or analysed during the current study are not publicly available due to ethical restrictions, patient privacy considerations, and/or institutional policies, but are available from the corresponding author on reasonable request

Author Contributions

SC, ESP, and SA designed the research study and wrote the first draft. SC, ESP and SA performed the research. SC and ESP analyzed the data. All authors contributed to important editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was approved by Çanakkale Onsekiz Mart University's Clinical Research Ethics Committee (Decision No: 2023/07-02, Date: May 03, 2023; Application No: 2023-46) and was carried out in compliance with the Declaration of Helsinki's ethical guidelines. Written informed consent was obtained from all participants prior to enrollment.

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Conflict of Interest

The authors declare no conflict of interest.

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