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Prevalence of genetic polymorphisms ABCB1/ C3435T in patients with epilepsy in Iranian population

Abstract

Introduction: Epilepsy is a common neurological disorder affecting approximately 1% of the global population. A significant proportion of patients develop drug-resistant epilepsy (DRE), which leads to poor outcomes for treatments and low quality of life. The multidrug transporter P-glycoprotein, encoded by the ABCB1 gene, plays a key role in limiting brain penetration of antiepileptic drugs. The C3435T single nucleotide polymorphism (SNP) in ABCB1 has been linked to variable P-glycoprotein expression and AED response. In this study, the prevalence of C3435T genotypes among epilepsy patients and healthy controls in Fars Province, Iran was investigated.

Materials and methods: This study involved 50 patients diagnosed with epilepsy and 100 healthy individuals without a history of seizures or epilepsy. Genotyping was conducted using PCR-RFLP analysis.

Results: The variant T allele occurred at a frequency of 52% in cases and 54% in controls. However, no significant association between the C3435T polymorphism and epilepsy risk was observed. Interestingly, it was found that the CT genotype was overrepresented in drug-responsive patients compared to those with drug-resistant cases. This suggests that individuals with the CT genotype may have a more favorable response to antiepileptic drug therapy.

Conclusion: These findings suggest that the ABCB1 C3435T variant may play a role in influencing seizure control specifically among Iranian patients, but its effects on susceptibility are minimal. Moreover, individuals with the CT genotype may have a more favorable response to antiepileptic drug therapy. Further research involving larger cohorts is needed to fully understand the impact of ABCB1 genetics on determining individual responses to antiepileptic drugs. Such research could also pave the way for personalized management strategies based on pharmacogenomic testing.

Keywords: epilepsy, ABCB1 C3435T, gene polymorphism, P-glycoprotein

Volume 6 Issue 1 - 2023

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Received: October 19, 2023 | **Published:** October 30, 2023

Introduction

Epilepsy is a pervasive neurological disorder that affects a substantial proportion of the global population, with approximately 50 million individuals suffering from this condition worldwide.[1] This disorder is characterized by recurring and unprovoked seizures resulting from abnormal electrical activity in the brain.[2] The prevalence of epilepsy stands at around 1% globally, although low- and middle-income countries tend to experience higher rates.[3] In Iran specifically, epidemiological investigations estimated 16.6 per every 1000 people within their population.[4] It is important to note that epilepsy not only causes increased morbidity and mortality but also imposes significant psychosocial as well as economic burdens upon affected individuals and society.[5,6] Antiepileptic medications (AEDs) are the primary form of treatment for epilepsy. Although most patients can manage their seizures with AEDs, about 36.3% of individuals eventually develop drug-resistant epilepsy (DRE).[7,8] The inability to establish prolonged seizure control despite adequate trials with two tolerated and carefully chosen AED regimens is referred to as drug-resistant epilepsy. It is critical to pay attention to the phenomena of DRE because it has serious repercussions, including the risk of premature death, damage, impaired psychosocial functioning, and poor quality of life.[9] DRE also poses a difficult problem whose underlying mechanisms are still not fully understood. However, researches are indicating that genetic factors contribute significantly to this disease.[7,10] One gene that has generated significant interest in the field is the multidrug resistance 1 (MDR1 or ABCB1) gene. P-glycoprotein (P-gp), which serves as an efflux transporter protein, is specifically encoded by this gene and is highly expressed in tissues with barrier function, including the blood-brain barrier, liver, intestines, and kidneys.[11] P-gp's primary function is to inhibit drug absorption and penetration into the brain via an active efflux transport mechanism.[12] Antiepileptic drug accumulation was found to be greater in the brains of P-gp knockout mice compared to wild-type mice in experiments, demonstrating the crucial function that P-gp plays in limiting AED penetration into the brain.[13] Furthermore, studies have discovered P-gp expression not only in astrocytes but also in capillary endothelial cells in drug-resistant epileptogenic brain tissue from patients. These findings point to a possible connection between this transporter protein and AED resistance.[14] More than 50 single nucleotide polymorphisms (SNPs) have been found in the MDR1 gene, which is extremely variable in humans.[15] Some of these SNPs have been linked to variations in the expression or function of P-gp, a drug transport protein. C3435T is a well-known SNP that occurs within a specific section of the gene known as exon 26 and does not modify the arrangement of amino acids it codes for. Individuals bearing the T variation initially had reduced levels of P-gp expression, particularly in the duodenum.[16] However, following research on its functional impact has shown contradictory results.[17] Nonetheless, many studies demonstrate that people with the 3435T allele had greater plasma concentrations of medicines that

are P-gp substrates, such as phenytoin and phenobarbital, which are routinely used antiepileptic medications.[18–20] This might indicate lower P-gp activity, which would result in less effective efflux transport and more of these drugs building up inside the brain. In a study conducted by Sadiq et al. on 115 patients with epilepsy, the potential importance of MDR1 polymorphisms in AED resistance was investigated. This study showed that the 3435CC genotype was significantly more frequent in patients with DRE compared to responders, suggesting that it may confer susceptibility.[21] Several subsequent studies yielded similar findings linking the 3435CC genotype to poorer response.[22–24] However, other studies found no association between 3435TT and poorer drug response.[22–26] A meta-analysis of 16 studies found no overall association between C3435T and drug-resistant epilepsy.[27] The discrepancies in the findings mentioned earlier can be attributed to variations in sample size and ethnic backgrounds among the studies, indicating that different ethnic groups may exhibit diverse outcomes. However, there has been a lack of investigation into MDR1 polymorphisms specifically among Iranian individuals with epilepsy. Considering Iran's genetic admixture from neighboring populations, it is likely that unique variants and haplotypes exist within this population. Consequently, associations observed in other Asian cohorts may not apply universally to Iranians. Given the high prevalence of epilepsy in Iran, research focusing on this population holds significant clinical and public health importance as it could provide insights into the underlying mechanisms of treatment resistance and potentially pave way for personalized therapeutic strategies tailored to Iranian patients with epilepsy. This study aims to determine the prevalence of the C3435T polymorphism in Iranian epilepsy patients compared to healthy controls in Fars province. It will also examine the association between different C3435T genotypes and drug response. Understanding the role of this variant in Fars population may provide insights into drug resistance mechanisms, which can then contribute to larger pharmacogenomic studies on epilepsy within this ethnic group. Ultimately, a comprehensive understanding of genetic factors influencing antiepileptic drug response could lead to molecular diagnostics that enable personalized therapy selection and improve outcomes for individuals with epilepsy.

Materials and methods

Study design and participants

This study involved 50 patients diagnosed with epilepsy and 100 healthy individuals without a history of seizures or epilepsy. The participants were recruited from the Neurology Clinic at Namazi Hospital, Shiraz University of Medical Sciences in Iran. Epilepsy diagnosis was based on clinical presentation, EEG findings, and neuroimaging studies. Patients with pseudo seizures or seizures caused by metabolic disorders were excluded from the study. The control group consisted of age- and sex-matched individuals who had no previous episodes of seizures or epilepsy. All participants provided written informed consent to participate in the study.

Sample collection and DNA extraction

After obtaining informed consent, a total of 3 mL of peripheral blood was gathered from each participant using EDTA tubes. Genomic DNA was isolated from the leukocytes present in the blood samples following a standard salting-out protocol as previously described.[28] The concentration and purity of the extracted DNA were assessed using spectrophotometry by measuring the A260/A280 ratio. Subsequently, the obtained DNA samples were stored at -20°C until they were ready for further analysis.

Genotyping of ABCB1 C3435T polymorphism

The genotyping of the ABCB1 C3435T polymorphism (rs1045642) was conducted using polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP).[23] The PCR step involved a 25 µL reaction mixture containing 100 ng of genomic DNA, 10 pmol of each primer, 2.5 mM dNTPs, 1X PCR buffer, and 1.5 mM MgCl2 along with Taq DNA polymerase at a concentration of 1 unit per reaction. To amplify the target region in the DNA samples, we utilized specific primers that were previously published. The thermocycling conditions consisted of an initial denaturation step at a temperature of 95°C for a duration of five minutes. This was followed by 35 cycles consisting of three steps: denaturation at 95°C for thirty seconds, annealing at 56°C for thirty seconds, and extension at 72 °C for thirty seconds. Finally, there was a final extension stage where the temperature remained constant at 72 °C for five minutes to ensure complete synthesis. The PCR products were subjected to enzymatic digestion using 2 units of the Mbo1 restriction enzyme and incubated at 37°C overnight. The resulting fragments were separated on a 3% agarose gel, together with a 100 bp DNA ladder for reference. The presence of bands at 163 bp and 68 bp indicated the wild-type C allele, whereas the variant T allele was represented by a single band at 231 bp. To ensure quality control, randomly chosen samples (10% of total) were selected for duplicate genotyping analysis, yielding consistent results with complete agreement between duplicates Figure 1.

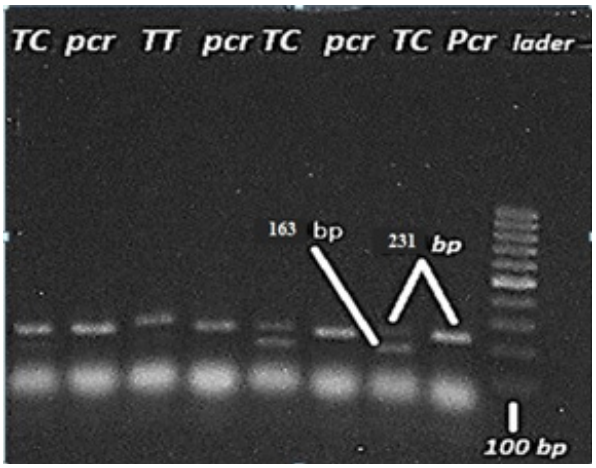

Figure 1 Cutting of the separated sections of the MDR1 gene by MBO1 enzyme; with this enzyme, TT olymorphism produces a 231 band, TC polymorphism produces a 231 band and a 163 band, and CC polymorphism produces a 163 band.

Statistical analysis

The data analysis was performed using SPSS version 21.0 from IBM Corp, located in Armonk, NY. The calculation of allele and genotype frequencies was done through direct counting. To compare the distribution of genotypes between cases and controls, Pearson's chi-square test was used. In order to evaluate the association between ABCB1 C3435T polymorphism and epilepsy risk, odds ratios along with their corresponding 95% confidence intervals were calculated. P values less than 0.05 were considered statistically significant.

Results

Patient characteristics

The current study involved the participation of a total of 150 individuals, including 50 patients who were clinically diagnosed with epilepsy and 100 healthy controls. Among the patient group, there

were 28 males and 22 females, with a mean age of 30.5±11.2 years. In comparison, the control group consisted of 52 males and 48 females, with a mean age of 29.8±10.6 years. Upon analyzing the data obtained from both groups, it was determined that no significant differences existed regarding age (p=0 .712) or gender (p=0 .789) between them. These findings indicate that the distribution of demographic characteristics was similar among epileptic patients and healthy individuals in this study.

Table 1 Demographic characteristics of epilepsy patients and healthy controls.

Parameter	Cases (n=50)	Controls (n=100)	P-value
Age (years), mean±SD	30.5±11.2	29.8±10.6	0.712
Gender, n (%)			
Male	28 (56%)	52 (52%)	0.789
Female	22 (44%)	48 (48%)	

ABCB1 C3435T genotype distribution

The genotyping of the ABCB1 C3435T polymorphism was performed on all participants using the polymerase chain reaction-restriction fragment length polymorphism analysis technique. The 231 bp PCR amplicon obtained was then subjected to digestion with Mbo1 restriction enzyme. This enzymatic treatment resulted in three different banding patterns visible on Figures 1 and 2, representing the CC, CT, and TT genotypes. In the sample of epilepsy patients analyzed, it was found that the prevalence of ABCB1 3435TT homozygous genotype was observed in 20% (10 out of 50 cases), while heterozygous CT and homozygous CC genotypes were observed in 64% (32 patients) and16% (8 patients) respectively. In comparison, among the healthy control group, frequencies for TT, CT, and CC genotypes were reported as 28%, 52%, and 20%. However, no statistically significant difference was identified when comparing C3435T genotypes distribution between epilepsy patients and controls (p=0.371). These findings indicate that there is no association between different C3435T genotypes with susceptibility to developing epilepsy. The occurrence frequency of the mutant T allele was relatively similar among both cases and controls, with percentages of 52% and 54% respectively (p=0.743). Taken together, our findings on genotype and allele distributions suggest that there is no significant association between the ABCB1 C3435T variant and epilepsy risk in this particular cohort from Iran. This finding adds to a growing body of evidence suggesting conflicting results regarding the relationship between the C3435T polymorphism of ABCB1 gene and susceptibility to epilepsy across different populations. Future studies should aim to clarify these inconsistencies through larger cohorts or more diverse ethnic groups.

Table 2 Distribution of ABCB1 C3435T genotype and allele frequencies in epilepsy patients and controls.

Genotype/Allele	Cases (n=50)	Controls (n=100)	P-value
Genotype, n (%)			
CC	8 (16%)	20 (20%)	0.371
CT	32 (64%)	52 (52%)	
TT	10 (20%)	28 (28%)	
Allele			
C	48 (48%)	92 (46%)	0.743
T	52 (52%)	108 (54%)	

Association with anti-epileptic drug response

We next stratified epilepsy patients based on their response to anti-epileptic drug (AED) therapy. Of the 32 patients with the ABCB1 3435CT genotype, 21 (65.6%) exhibited good seizure control with AEDs. In contrast, only 2 of 8 CC homozygotes (25%) and 3 of 10 TT homozygotes (30%) were classified as drug-responsive. Notably, the CT genotype was significantly overrepresented among drug-responsive patients compared to treatment-resistant cases (p=0.036). These preliminary findings suggest ABCB1 C3435T genotype may correlate with variable sensitivity to AEDs in epilepsy patients, with the CT variant associated with a favorable treatment response.

Table 3 Association between ABCB1 C3435T genotype and anti-epileptic drug (AED) response in epilepsy patients.

Genotype	AED Responsive	AED Resistant	P-value
CC	2 (25%)	6 (75%)	0.036
CT	21 (65.6%)	11 (34.4%)	
TT	3 (30%)	7 (70%)	

Discussion

In this case-control study, we did not detect any significant association between the ABCB1 C3435T polymorphism and epilepsy susceptibility in an Iranian population. The variant T allele occurred at similar frequencies of 52% and 54% among cases and controls, respectively. Additionally, the distribution of CC, CT, and TT genotypes did not differ significantly between patients and healthy individuals. These results concur with several previous studies in Asian cohorts that found no correlation between genotype or allele frequencies at this locus and epilepsy risk.[29,30] However, one study including 115 epilepsy patients reported a modest but statistically significant association between CC genotype and drug-resistant epilepsy.[21] The lack of replication in our dataset may be attributed to the limited sample size as well as the heterogeneity underlying epilepsy, which is likely influenced by complex interactions among various genetic and environmental factors. Notably, our preliminary findings indicate the ABCB1 3435CT genotype may be associated with better seizure control in response to anti-epileptic drug therapy. The CT heterozygous patients exhibited a significantly higher rate of treatment responsiveness (65.6%) compared to CC (25%) and TT (30%) homozygotes. A favorable impact of the T allele on drug response aligns with previous evidence that the 3435T variant is correlated with enhanced brain accumulation of AEDs which are P-gp substrates. Reduced P-gp expression or efflux function linked to this allele could enable sufficient therapeutic levels of AEDs to be attained within the brain. However, conflicting reports exist, with several studies finding no influence of C3435T polymorphism on drug resistance.[31] Furthermore, a comprehensive meta-analysis conducted on the subject has suggested that ABCB1 variants may have a minimal impact on the overall variability of anti-epileptic drug response.[27] The discrepant findings may reflect differences in study design, sample size, ethnicity, or the criteria used to define drug-resistant epilepsy across studies. Furthermore, resistance likely arises from a multifactorial interplay among genes encoding drug-metabolizing enzymes, transporters, and targets, rather than single-gene effects.[32] Additional research in expanded cohorts is warranted to clarify the relationship between ABCB1 genotype and treatment outcomes in epilepsy, which could facilitate pharmacogenomic testing to guide personalized therapeutic management. In summary, we did not establish a significant association between ABCB1 C3435T polymorphism and epilepsy susceptibility among Iranians. However, our preliminary findings raise the possibility that this variant may influence response to anti-epileptic medications. Further investigation of the role of ABCB1 genetics in variable drug effects is needed and may eventually enable individualized therapy selection to improve outcomes for patients with epilepsy.

Citation: Miladpour B, owji M, Mokhtari MM. Prevalence of genetic polymorphisms ABCB1/C3435T in patients with epilepsy in Iranian population. *Int J Mol Biol Open Access*. 2023;6(1):54–57. DOI: 10.15406/ijmboa.2023.06.00152

Acknowledgments

We greatly acknowledge Fasa University of Medical Sciences for funding and supporting us in this study. Grant NO. 95114

Conflicts of interest

The authors declare that there are no conflicts of interest.

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