**ABCB1 C3435T Gene Polymorphism Frequency and Correlation with Clinical Parameters in Multiple Sclerosis**

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**Abstract**

**Background:** People with Multiple Sclerosis (MS) show varying responses to the same drugs, suggesting a genetic factor. In addition, certain ABCB1 gene polymorphisms have been associated with resistance to many drugs. In the era of individualized treatment, identifying possible genetic causes of drug nonresponsiveness in MS patients may enable the prediction of nonresponse before treatment. Research is needed to determine relationships between ABCB1 polymorphisms and patients' clinical parameters and drug response in MS.

**Objective:** This study investigated the presence of ABCB1 C3435T polymorphism among patients with MS and evaluated possible associations between C3435T variants and disease activity and clinical parameters in MS.

**Materials and Methods:** The study included 100 patients aged 18 and over who were definitively diagnosed with MS according to the 2010 McDonald diagnostic criteria and were receiving immunomodulatory therapy, as well as a group of 100 healthy individuals. Clinical and demographic characteristics of the MS group were recorded. All study participants underwent ABCB1 C3435T genotyping. A blood sample was collected from each participant and used for DNA isolation and single-nucleotide polymorphism analysis.

**Results:** There was no statistically significant difference between MS patients and the healthy subjects with regard to ABCB1 C3435T variants. Mean score on the Extended Disability Status Scale was significantly higher in MS patients with the CT variant of the ABCB1 polymorphism compared to those with CC and TT variants, indicating that disability was more severe in MS patients with the CT genotype of the ABCB1 C3435T polymorphism.

**Conclusion:** Considering the role of P-glycoprotein in drug pharmacokinetics, the results of this study suggest a possible benefit of assessing MS patients for ABCB1 gene polymorphisms. The literature includes very little information available regarding its relevance in MS. Therefore, the present study evaluated associations between the ABCB1 C3435T polymorphism and MS patients' clinical parameters such as disease activity and drug changes. The results showed that the heterozygous CT genotype of ABCB1 was associated with significantly higher disability scores, as well as a trend toward higher annual relapse rate and fewer drug changes. This suggests that ABCB1 may have an important role in MS that warrants further research in order to make progress toward individualized therapy for people with MS.

**Keywords:** ABCB1 gene; C3435T; Disability; Multiple sclerosis; Polymorphism

**Statement of Significance**

Multiple Sclerosis (MS) is a debilitating and heterogeneous disease whose management continues to present major challenges. People with MS exhibit a wide range of clinical presentations as well as varying responses to the same drugs, which both indicate that genetic factors are involved in disease course and treatment response. The ability to predict treatment response in MS patients would prevent unnecessary drug usage, limit adverse side effects, and enable earlier direction of patients to effective treatment methods. The ABCB1 gene, which encodes P-glycoprotein 1, has been associated with resistance to many drugs. While the literature includes studies investigating the role of ABCB1 polymorphisms in other autoimmune diseases, there is little information available regarding its relevance in MS. Therefore, the present study evaluated associations between the ABCB1 C3435T polymorphism and MS patients' clinical parameters such as disease activity and drug changes. The results showed that the heterozygous CT genotype of ABCB1 was associated with significantly higher disability scores, as well as a trend toward higher annual relapse rate and fewer drug changes. This suggests that ABCB1 may have an important role in MS that warrants further research in order to make progress toward individualized therapy for people with MS.

**Introduction**

Multiple Sclerosis (MS) is an autoimmune inflammatory demyelinating neurodegenerative disease of Central Nervous System (CNS) with relapsing-remitting and progressive forms [1]. MS is more common in young adults and women. Age of onset is between 20 and 40 in approximately 65% of patients. Prevalence studies have shown that the incidence is 2-3 times higher among women than men [2]. The prevalence of MS, which is known to be affected by genetic and environmental factors, varies in different regions. It is considered that this variation is caused by different seasonal characteristics, geographic locations, and ethnic profiles of the population [3]. Although the prevalence and incidence of MS in Turkey have not been definitively determined, it is likely a medium-risk region. A prevalence of 101.4 per 100,000 was reported based on clinical observations [4]. Although its etiology remains unclear, MS is believed to be affected by genetic, environmental, viral, and autoimmune factors [5].

Because inflammation in MS can appear throughout the brain,
spinal cord, and optic nerve, it can manifest with any symptom related to the central nervous system. Common signs and symptoms include weakness in the extremities, sensory symptoms, ataxia, bladder problems, fatigue, visual symptoms such as diplopia and blurred vision, dysarthria, and cognitive symptoms such as memory, concentration, and attention disorders [6].

MS is currently diagnosed using the McDonald criteria. These criteria were first introduced in 2001 and revised in 2005, 2010, and 2017. Diagnosis is made based on clinical symptoms and findings. Supportive laboratory findings are obtained through analysis of Magnetic Resonance Imaging (MRI), Cerebrospinal Fluid (CSF), and Visual Evoked Potentials (VEP) [7-9].

MS has several forms which vary in terms of disease course. The most common form encountered in clinical practice is Relapsing Remitting MS (RRMS). Approximately 80%-90% of patients present with RRMS [10]. Primary Progressive MS (PPMS), which accounts for about 10%-15% of cases, is characterized by progressive worsening of symptoms without acute relapses [11]. In Secondary Progressive MS (SPMS), neurodegeneration is more predominant than the inflammatory process. Seventy-five percent of RRMS patients naturally progress to SPMS [12]. An extremely small proportion of patients exhibit a chronically progressive course with acute relapses but no recovery between, a form called Relapsing Progressive MS (RPMS) [13,14].

There is currently no treatment to cure or prevent MS. Treatment options can be grouped into four subheadings: treatment of acute relapses, symptomatic treatment, disease-modifying therapy, and rehabilitation. Acute relapses are treated using corticosteroids and, less frequently, Adrenocorticotropic Hormone (ACTH). Disease-Modifying Therapies (DMTs) including immune-modulatory and immunosuppressant drugs delivered by injection, infusion, and oral route to slow down the progression of MS and to improve patients’ quality of life [1-10]. Most of these drugs were approved for the treatment of RRMS. Only mitoxantrone is also approved for the secondary progressive and relapsing progressive forms and ocrelizumab for PPMS.

Patients treated with Interferon-beta (IFN-β) and natalizumab may develop Neutralizing antibodies (NAbS) that can adversely affect therapeutic response. A study revealed that MS patients treated with IFN-β who had persistent NAb positivity exhibited more frequent disease activity [15].

Treatment is less effective in NAb-positive patients. Other than this factor, there is no other predictive indicator for DMT response. Identifying the reasons underlying nonresponse to DMTs may serve as possible predictors of treatment failure [15].

Carrier proteins are very large protein families that transport various drugs, xenobiotics, and endogenous compounds across membranes. They have recently attracted attention because of their role in antineoplastic drug resistance and their effects on drug pharmacokinetics [16].

Inter-individual variations in drug responses are attributed to polymorphisms or rare phenotypes [19]. The carrier protein that best demonstrates the effect of polymorphisms on pharmacokinetics is P-gp, encoded by the ABCB1 gene. Of the 29 Single-nucleotide Polymorphisms (SNPs) identified for ABCB1, C3435T in exon 26 and G2677T in exon 21 alter the substrate specificity of P-gp to eliminate its carrier protein function. The C3435T and G2677T SNPs may show ethnic variation and imbue resistance to drugs that are substrates of P-gp, thus influencing treatment responses and the prevalence of some diseases [20].

Studies have demonstrated a possible correlation between ABCB1 polymorphism and non-responsiveness to certain drugs. There are also numerous studies regarding ABCB1 polymorphism in relation to autoimmune diseases, immunosuppressant drugs used after transplantation, the determination of psychiatric drug levels, and non-responsiveness to drugs [16,21-23].

In light of these data, this study was conducted to determine the frequency of the ABCB1 C3435T polymorphism in 100 MS patients being treated with various drugs with different mechanisms of action, and compare them with those of 100 healthy controls. The objective of the study was to identify potential correlations between C3435T variants and disease activity and clinical parameters in MS patients.

Materials and Methods

This study was jointly planned by the Medical Biology and Neurology Departments of Ataturk University in Erzurum, Turkey. One hundred MS patients aged 18 and over who presented to the MS outpatient clinic of the Erzurum Ataturk University Medical School Neurology Department were included in the study. All patients were diagnosed according to the 2010 McDonald diagnostic criteria and were being treated with various drugs with different mechanisms of action, including immunosuppressant drugs used after transplantation, the determination of psychiatric drug levels, and non-responsiveness to drugs [16,21-23].

In light of these data, this study was conducted to determine the frequency of the ABCB1 C3435T polymorphism in 100 MS patients being treated with various drugs with different mechanisms of action, and compare them with those of 100 healthy controls. The objective of the study was to identify potential correlations between C3435T variants and disease activity and clinical parameters in MS patients.

Genomic DNA extraction

Genomic DNA isolation was done using whole blood samples (2cc) collected from each patient into EDTA tubes) using a commercial kit (EZ1 DNA Blood Kit: Qiagen, Germany). DNA was extracted from 200 μL aliquots of whole blood according to the manufacturer’s protocol. DNA concentration was diluted to 10 ng/μL for working solutions and the isolated DNA was stored at -20°C.

ABCB1 polymorphism analysis

The present study focused on one Single-nucleotide Polymorphism (SNP) of the ABCB1 gene: C3435T (rs1045642). Polymerase Chain Reaction (PCR) and melting curve analyses were performed under the same conditions in a 96-well plate using a Light Cycler 480 (Roche Diagnostics, Penzberg, Germany). Genotyping was done with Light SNP typing assay (TIB-MolBiol, Berlin, Germany) by analyzing melting curves with the LightCycler 480 II system (Roche Applied Science, Mannheim, Germany). Samples with a final volume of 20 μL were
prepared by combining 2 μL of purified genomic DNA (~50 ng), 2 μL of Fast Start DNA Master HybProbe (Roche Diagnostics, Mannheim, Germany), 1 μL of Light SNiP Reagent Mix (TIB-MolBiol, Berlin, Germany), 1.6 μL of 25 mM MgCl2, and 13.4 μL of distilled H2O. Real-time PCR was performed as follows: denaturation at 95°C for 10 min, followed by 45 cycles of 95°C for 10 s, 60°C for 10 s, and 72°C for 15 s. After the amplification phase, a melting curve analysis was performed at 95°C for 30 s, 40°C for 2 min, 75°C for 0 s, followed by cooling phase at 40°C for 30 s. Collected data were analysed using LightCycler 480 Gene Scanning software version 1.2 (Roche Diagnostics).

Statistical Analysis
The study data were analysed using SPSS® version 23.0 (IBM Corp., Armonk, NY, USA) statistical software package. Frequency distribution, mean, and standard deviation values were used for comparisons between the groups. Kolmogorov-Smirnov test was used to assess normality of data distributions. Kruskal-Wallis variance of analysis of variance was used for comparison of Extended Disability Status Scale (EDSS) scores because the data were normally distributed. ANOVA was used for comparison of Extended Disability Status Scale (EDSS) scores because the data were normally distributed. Paired comparisons between groups showed statistically significant difference in EDSS between the control and treatment groups.

Results
The study included 100 MS patients and 100 healthy individuals. Frequencies of ABCB1 C3435T variant genotypes and allele frequencies in MS patients and controls are presented in Table 1. The mean age of the patients was 36.41 ± 9.53 years and the male to female ratio was 1:2.6. Frequencies of ABCB1 variants in the patient and control groups are summarized in Table 1. In the RRMS group, the heterozygous CT genotype was the most common, present in 38 patients, followed by the ho Of the MS patients who were included in the study group, 63 used DMT drugs (Interferon beta1a, Interferon beta1b, Glatiramer Acetate), 30 oral (Teriflunamide, Dimethyl Fumarate, Fingolimod) treatment and 7 patients were using Natalizumab (300 mg, iv). When the relationship between drug groups and ABCB1 polymorphism 3435C>T was examined, all of the treatment groups had more CT variants. Mozgyous TT genotype in 28 patients and homozygous CC genotype in 21 patients. The patients' demographic and clinical characteristics and drugs used are presented in Table 2.

The MS patients were compared by ABCB1 C3435T genotype in terms of EDSS score, age at disease onset, duration of disease, annual relapse rate, and number of drug changes (Table 3). Annual relapse rate was highest among the MS patients with the CT genotype (0.97 ± 0.01), but the difference between the groups was not statistically significant (p<0.05) (Figure 1) (Table 4). Number of drug changes was lowest in patients with the CT genotype and highest in those with the CC genotype, but there was no statistically significant difference between the groups (p<0.05) (Figure 1). Mean EDSS scores by polymorphism group were 2.48 in CT, 1.89 in TT, and 1.80 in CC. EDSS was significantly correlated with polymorphism (p<0.05). Paired comparisons between groups showed statistically significant differences in EDSS between the CT and CC groups and between the CT and TT groups (p<0.05).

Discussion
Although its etiology of MS is not well understood, it is considered

Table 1: Distribution of C3435T genotypes and allele frequencies in MS patients and controls. *MS: Multiple sclerosis.

<table>
<thead>
<tr>
<th>ABCB1 3435C&gt;T Haplotype</th>
<th>MS* (n)</th>
<th>Control (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>23</td>
<td>21</td>
</tr>
<tr>
<td>CT</td>
<td>46</td>
<td>40</td>
</tr>
<tr>
<td>TT</td>
<td>31</td>
<td>34</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2: Demographic and clinical characteristics of multiple sclerosis patients genotyped for ABCB1.

<table>
<thead>
<tr>
<th>Type of multiple sclerosis</th>
<th>TT (n=31)</th>
<th>CC (n=23)</th>
<th>CT (n=46)</th>
<th>Total (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>1:4.43</td>
<td>1:2.55</td>
<td>1:3.83</td>
<td>1:2.6</td>
</tr>
<tr>
<td>Age, years (mean ± SD)</td>
<td>35.13 ± 8.10</td>
<td>38.22 ± 11.69</td>
<td>36.37 ± 9.30</td>
<td>36.41 ± 9.53</td>
</tr>
<tr>
<td>Age at onset, years (mean ± SD)</td>
<td>30.95 ± 8.20</td>
<td>31.96 ± 9.17</td>
<td>28.78 ± 9.00</td>
<td>30.19 ± 8.81</td>
</tr>
<tr>
<td>Duration of disease, years (mean ± SD)</td>
<td>4.18 ± 2.18</td>
<td>6.26 ± 5.79</td>
<td>7.59 ± 6.54</td>
<td>6.23 ± 5.34</td>
</tr>
<tr>
<td>Annual relapse rate (mean ± SD)</td>
<td>0.86 ± 0.01</td>
<td>1.02 ± 0.03</td>
<td>0.97 ± 0.01</td>
<td>0.88 ± 0.02</td>
</tr>
<tr>
<td>Number of drug changes (mean ± SD)</td>
<td>0.46 ± 0.01</td>
<td>0.49 ± 0.01</td>
<td>0.42 ± 0.01</td>
<td>0.45 ± 0.01</td>
</tr>
<tr>
<td>EDSS (mean ± SD)</td>
<td>1.89 ± 0.01</td>
<td>1.80 ± 1.31</td>
<td>2.48 ± 1.32</td>
<td>2.14 ± 1.26</td>
</tr>
<tr>
<td>Type of multiple sclerosis</td>
<td>28</td>
<td>21</td>
<td>38</td>
<td>87 (87%)</td>
</tr>
<tr>
<td>RRMS</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>12 (12%)</td>
</tr>
<tr>
<td>SPMS</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1 (1%)</td>
</tr>
</tbody>
</table>

Table 3: Drugs used by MS patients as disease-modifying therapy.
levels was found and found that gene polymorphisms affect drug blood levels by determining that drug blood level (digoxin) is higher in patients with TT genotype [26].

Kerb et al. suggested that ABCB1 polymorphism is an important parameter in the development of drug resistance [27]. The C3435T polymorphism is the most common ABCB1 gene polymorphism, resulting from a single base change (C-T) at position 3435 in exon 26. It does not cause an amino acid change [28].

The effects of ABCB1 SNPs are a newly emerging area of research in MS etiology. No statistical differences have been previously reported between MS patients and controls in terms of ABCB1 2677G>T and 3435C>T. SNPs in ABC transporter genes can function as pharmacogenetic markers associated with clinical response to drug therapy in multiple sclerosis [29].

In the present study, the mean age at MS onset was 19 years, and the patients’ mean age was 36.4 years. The male to female ratio was 1:2.6, which is consistent with the literature. Similarly, the most common form of MS in the present study was RRMS, accounting for 87% of the patients. An additional 12% of the patients had SPMS and 1% had PPMS.

**Conclusion**

Our study constitutes a small population of MS patients in Turkey. The results of this study show that EDSS values were significantly higher among patients with the CT polymorphism when compared with the other two groups. This suggests that disability is more severe in MS patients having the CT genotype of the ABCB1 polymorphism. Presence of CT polymorphism more extensive and large studies are needed to determine whether MS patients are a predictive parameter for progression. This study may contribute to the literature in that sense. Subsequent studies will investigate larger populations with a broader scope.

**Author Contributions**

Study design/planning: Eda Balkan; Data collection/entry: Nuray Bilge; Data analysis/statistics: Nuray Bilge; Data interpretation: Eda Balkan; Manuscript preparation: Eda Balkan; Literature search/analysis: Eda Balkan; Funds collection: Eda Balkan and Nuray Bilge.

**Ethical Standards**

The study was conducted in compliance with international, national, and institutional regulations. The Ataturk University Medical Faculty Ethics Committee approved the study. All persons provided informed consent prior to inclusion in the study.

**References**

Citation: Balkan E, Bilge N (2018) ABCB1 C3435T Gene Polymorphism Frequency and Correlation with Clinical Parameters in Multiple Sclerosis. J Neurol Neurophysiol 9: 479. doi:10.4172/2155-9562.1000479


