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Statin and MTHFR C677T Polymorphism in Patients with Cardiovascular Diseases

Statin Kullanan Kardiyovasküler Sistem Hastalarında MTHFR C677T Polimorfizmi

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ABSTRACT

Objective: Cardiovascular disease (CVD) is the leading cause of death worldwide. The methylenetetrahydrofolate reductase (MTHFR) gene, located on the short (p) arm of chromosome 1 at position 36.3 (1p36.3), might be a possible risk factor for the pharmacogenetics in CVD. A common polymorphism in *MTHFR* (C677T, Ala→Val) decreases this enzyme activity and increases the homocysteine concentrations, predisposing one to heart disease. Alternatively, statins, cholesterol-reducing agents, are also used to reduce the homocysteine blood concentrations; the aim of the present study was to evaluate how the genotype frequencies of the *MTHFR* C677T polymorphism, namely rs1801133, change in the cardiovascular system in patients treated with statin.

Methods: In this study, the genotype distribution of the *MTHFR* C677T polymorphism in CVD patients treated with statin (hydrophilic and lipophilic) (n=290) and healthy controls (n=151) was assessed using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP).

Results: In this study, a statistically significant difference in genotype frequencies for the *MTHFR* C677T polymorphism was found between CVD patients treated with statin and controls (p=0.037).

Conclusion: For the first time, we demonstrate a relation between a *MTHFR* gene polymorphism and CVD in patients treated with statins in the Turkish population.

Key Words: MTHFR, cardiovascular disease, statin, pharmacogenetics

ÖZET

Amaç: Kardiyovasküler hastalıklar dünyada ölüm nedenlerinin başında gelmektedir. Kardiyovasküler hastalıkların farmakogenetiğinde muhtemel bir risk faktörü olan metilentetrahidrofolat redüktaz (MTHFR) enzimi, birinci kromozomun kısa (p) kolunun 36,3 bölgesinde (1p36.3) lokalize olan *MTHFR* geni tarafından kodlanmaktadır. *MTHFR* geninde yaygın olan C677T (Ala→Val) polimorfizmi *MTHFR* enziminin aktivitesini azaltarak kalp hastalıklarına yatkınlığı artıran homosistein konsantrasyonunu artırır. Lipid düşürücü olarak kullanılan statinler aynı zamanda kandaki homosistein konsantrasyonunu da düşürmektedir. Bu çalışmada statin ile tedavi edilmekte olan kardiyovasküler sistem hastalarında *MTHFR* C677T polimorfizmi (rs1801133) genotip dağılımlarının araştırılması amaçlanmaktadır.

Yöntemler: Bu çalışmada, lipofilik ve hidrofilik statinler ile tedavi edilen kardiyovasküler sistem hastalarında (n=290) ve sağlıklı kontrollerde (n=151) *MTHFR* C677T polimorfizmindeki genotip dağılımları incelenmiştir. Genotipleme, Polimeraz zincir reaksiyonu (PCR) ve restriksiyon parça uzunluk polimorfizm (RFLP) yöntemi ile yapılmıştır.

Bulgular: Bu çalışmada, *MTHFR* C677T polimorfizminin genotip dağılımında statin tedavisi gören kardiyovasküler sistem hastaları ile kontrol grubu arasında istatistiksel olarak anlamlı bir fark gözlenmiştir (p=0,037).

Sonuç: Türk popülasyonunda statin ile tedavi edilen kardiyovasküler sistem hastaları ile *MTHFR* C677T polimorfizmi arasında istatistiksel olarak anlamlı bir ilişki ilk kez gösterilmiştir.

Anahtar Sözcükler: MTHFR, Kardiyovasküler Hastalıklar, Statin, Farmakogenetik

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Introduction

Cardiovascular disease (CVD) is the leading cause of death in Turkey (1). The conventional risk factors for CVD include genetic effects, hyperhomocysteinemia, smoking, hyperlipidemia, hypertension (HT), age, family history, diabetes mellitus (DM), and obesity (2).

Homocysteine, an amino acid intermediate in the conversion of methionine to cysteine, has been recognized as a new emerging risk factor for CVD (3). Methylenetetrahydrofolate reductase (MTHFR), a key enzyme in homocysteine metabolism (4), catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a cosubstrate for methylation of homocysteine to methionine (5). The *MTHFR* gene is located at the end of the short arm of chromosome 1 (1p36.3) (6). One of the well-described single-nucleotide polymorphisms of *MTHFR* is the C677T polymorphism, which stands in exon 4 and results in turning an alanine into valine at codon 222. This amino acid alteration causes an approximately 60% decrease in MTHFR enzyme activity compared to the wild-type *in vitro*. Therefore, MTHFR deficiency often results in elevated plasma homocysteine levels (7).

Pharmacogenetics is the study of how genetic factors affect inter-individual differences to treatment (8), and statin therapy has been largely investigated pharmacogenetically. Statins are a class of drugs used to lower cholesterol levels (9), and they also cause small reductions (3.5%) in homocysteine blood concentrations (10). The previous studies clearly showed that statins are effective for treating cardiovascular disease by lowering cholesterol and homocysteine in the blood. Inter-individual variation in response to statins (hydrophilic and lipophilic) is not sufficient to predict clinical benefit and adverse effects. As a consequence, more credible biomarkers are required for determining the sub-populations that may attain the most benefit from statin (hydrophilic or lipophilic) usage. However, as an individual therapy, the effect of statins in CVD is completely unclear. So, additional studies are needed to identify the contribution of individual statin therapies for CVD.

In the present study, we hypothesized that statins reduce homocysteine levels that are elevated by MTHFR enzyme deficiency due to *MTHFR* polymorphisms (rs1801133, C677T, Ala222Val). Thus, we investigated the relation of the C677T polymorphism in CVD patients under statin therapy and control groups in the Turkish population.

Materials and Methods

The study population consists of patients who diagnosed with CVD (n:290) under statin therapy at the Department of Cardiology (Bezmialem Vakif University) and healthy controls who came to the hospital for routine examination (n:151). This study and all experimental procedures were approved by the ethical committee of Bezmialem Vakif University. Written informed consent was obtained from each participant. Three milliliters of venous blood was taken from subjects to blood tubes with EDTA. DNA isolation of the blood samples collected from both groups was performed by a precipitation method using a commercial DNA isolation kit (Invitrogen). The *MTHFR* C677T polymorphism was analyzed by PCR-RFLP methods as previously described (11). Briefly, the 198-bp PCR products were digested with *Hinf I* for 3 hours at 37°C. Finally, genotypes were assessed on a 10% polyacrylamide gel.

Statistical analysis

The analysis of data was performed by the SPSS (11.5 version) program. To compare the ratios, the data were statistically analyzed by Pearson chi-square test. A 'p' value less than or equal to 0.05 was considered statistically significant.

Results

In the current study, we firstly determined the *MTHFR* C677T polymorphism in CVD patients under statin therapy. Initially, study groups were divided into three categories: a CVD group using lipophilic statin (lipo-statin), hydrophilic statin (hydro-statin), and a non-treatment (negative) control group. The frequencies of CC, CT, and TT variants were 40.8%, 46.1%, and 13.1% for non-treatment; 47.7%, 39.6%, and 12.6% for the lipophilic statin group; and 40.9%, 50%, and 9.1% for the hydrophilic statin group, respectively. There was no statistically significant relation between statin therapy in CVD patients and *MTHFR* C677T variants (p=0,720).

The *MTHFR* C677T polymorphism was in Hardy-Weinberg equilibrium in CVD patients and the control group. The allele frequencies of C and T in CVD patients treated with statin were 62% and 38%, respectively, and the allele frequencies regarding each of two alleles in the control group were 70% and 30%, respectively (Table 1). According to the Pearson chi-square test, a statistically significant difference in the frequencies of the T allele was demonstrated between the CVD patients and controls

Table 1. The frequencies of C/T allele and genotype for MTHFR C677T polymorphism

		CVD n (%)	Control n (%)	p value	OR CI95%
Allele Frequencies	C	361 (62%)	212 (70%)	0.023	1.43
1.06-1.93					
	T	219 (38%)	90 (30/%)		
Total		580 (100%)	302 (100%)		
Genotype					
	CC	111 (38.3%)	77 (51%)	0.037	
	CT	139 (47.9%)	58 (38.4%)		
	TT	40 (13.8%)	16 (10.6%)		
Total		290 (100%)	151 (100%)		

CVD: Cardiovascular Diseases; n: number of patients; OR: odds ratio; CI: Confidence interval

for the studied polymorphism (OR=1.43; CI 95%= 1.06-1.93; p=0.023; χ^2 =5.181). The genotype frequencies of the CC, CT, and TT variants in the CVD patients were 38.3%, 47.9%, and 13.8% for CVD patients under statin therapy and 51%, 38.4%, and 10.6% for the control group, respectively. According to the Pearson chi-square statistical method (Table 1), there was a significant difference between the genotype frequencies of CVD patients and the *MTHFR* C677T polymorphism (p=0.037).

The *MTHFR* C677T genotype frequencies according to the presence (doing exercise at least 3 times in a week for 30 min) or absence of exercise was also investigated. The genotype frequencies of CC, CT, and TT were 42.8%, 46.7%, and 10.5% for the negative exercise group and 37.5%, 47.2%, and 15.3% for the positive exercise group , respectively. However, the effect of exercise on the *MTHFR* C677T variants was insignificant (p=0.470).

Discussion

In the present study, we tested the hypothesis that the *MTHFR* C677T polymorphism is associated with CVD patients treated with statin (hydrophilic or lipophilic). When we analyzed the *MTHFR* gene polymorphisms, there were significant relations between the aforementioned polymorphism and CVD under statin therapy. However, we found that the *MTHFR* C677T polymorphism was not associated with differential statin efficacy. Additionally, in the present study, exercise has a protective role for the risk of CVD, even with possessing the mutant TT genotype.

In the literature, the relation between the *MTHFR* C677T polymorphism and the risk of CVD remains a controversial issue. Some previous studies reported a considerable difference between CVD patients and controls (12-15), while others had no difference (16-18).

It is known that cholesterol biosynthesis is inhibited by statins, limiting HMG-CoA reductase enzyme. Moreover, the action of statin is affected by *MTHFR*. In the literature, it was shown that the *MTHFR* C677T polymorphism causes high levels of homocysteine, which can be decreased by statins (1,6). Recent studies suggested that multiple genetic factors, especially *MTHFR*, which plays a role in the methylation pathway, act for personal therapy (19,20). However, in the present study, we could not find any relationship between the studied polymorphism and impact of statin.

The most important limitation of this study is that data on homocysteine values were not determined. Previous studies proved that *MTHR* polymorphisms elevate plasma homocysteine levels (21-23). On the other hand, statins cause small reductions (3.5%) in homocysteine blood concentrations (10).

Conclusion

This is the first report on the genotype and allele frequencies regarding the *MTHFR* C677T polymorphism in CVD patients under statin therapy and control subjects in the Turkish population. Statins, the most commonly prescribed lipid-lowering drugs, are generally effective for decreasing mortality in people with CVD. However, the efficiency of these agents differs from person to person due to comprehensive variability of genetic modifications. To predict the individual responses to drug therapies, the genetic differences of candidate molecules in metabolic mechanisms must be investigated**.** Further, molecular studies related with the association of the *MTHFR* C677T polymorphism and statin are needed for CVD individual treatment.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Bezmialem Vakif University (01.02.2012-7/15).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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