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# Thrombophilic gene variants in patients with coronary artery disease and myocardial infarction

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**ABSTRACT**

Coronary artery disease (CAD) and myocardial infarction (MI) are cardiovascular diseases that occur due to atherosclerosis (plaque formation) or atherosclerotic obstruction of the coronary arteries. Their genetic basis has been under investigation for a long time, and common variant studies link different genetic loci with these diseases. In this study, we investigated the possible association of coronary artery disease and myocardial infarction with thrombophilic gene variants, including MTHFR C677T and A1298C, Beta fibrinogen -455G/A, Factor XIIIV34L and PAI-1 4G/5G single nucleotide polymorphisms (SNPs).

A total of 128 people (64 patients and 64 controls) were included in the study. Genomic DNA was isolated using the EZ1 blood mini kit. The DNA was amplified and PCR was performed using the PyroMark PCR Kit (Qiagen, Germany). Pyrosequencing reaction was completed by processing with PyroMark Q24 instrument.

We found that the PAI-1 4G/5G polymorphism and the 4G allele were significantly associated with coronary artery disease and myocardial infarction (P= 0.01). Although mutant variants were higher in patients, no statistically significant difference was observed between the patient and control groups in terms of FXIII, Beta-fibrinogen and MTHFR variants.

It is clear that the PAI-1 4G allele and the 4G/4G genotype have a significant contribution to the development of coronary artery disease and ultimately myocardial infarction. Prophylactic treatment should be considered in patients with this variant.

**Keywords**: Coronary artery disease, myocardial infarction, PAI-1, MTHFR, Beta fibrinogen, Factor XIII.

# Koroner arter hastalığı ve miyokard enfarktüsü olan hastalarda trombofilik gen varyantları

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**ÖZ**

Koroner arter hastalığı (KAH) ve miyokard infarktüsü (MI), aterosklerozun (plak oluşumu) veya koroner arterlerdeki aterosklerotik tıkanıklığın bir sonucu olarak ortaya çıkan kardiyovasküler hastalıklardır. Bunların genetik temeli uzun süredir araştırılmakta olup, yaygın varyant çalışmaları bu hastalıklarla farklı genetik lokusları ilişkilendirmektedir. Bu çalışmada, metilentetrahidrofolat redüktaz (MTHFR) C677T ve A1298C, Beta fibrinojen -455G/A, Faktör XIIIV34L ve PAI-1 4G/5G tek nükleotid polimorfizmleri (SNP'ler) de dahil olmak üzere trombofilik gen varyantlarının koroner arter hastalığı ve miyokard infarktüsü ile olası ilişkisi araştırıldı.

Toplamda 128 kişi (64 hasta ve 64 kontrol) çalışmaya dahil edildi. Genomik DNA, EZ1 blood mini kit kullanılarak izole edildi. DNA çoğaltıldı ve PCR, PyroMark PCR Kit (Qiagen, Almanya) kullanılarak gerçekleştirildi. Pyrosequencing reaksiyonu, PyroMark Q24 enstrümanı ile işlenerek tamamlandı.

PAI-1 4G/5G polimorfizmi ve 4G alleli'nin koroner arter hastalığı ve miyokard infarktüsü ile anlamlı bir şekilde ilişkili olduğunu bulduk (P= 0.01). Hasta gruplarında mutant varyantlar daha yüksek olmasına rağmen, hasta ve kontrol grupları arasında FXIII, Beta-fibrinojen ve MTHFR varyantları açısından istatistiksel olarak anlamlı bir fark gözlemlenmedi.

PAI-1 4G alleli ve 4G/4G genotipinin koroner arter hastalığının gelişimine ve nihayetinde miyokard infarktüsüne önemli bir katkısı olduğu açıktır. Bu varyanta sahip hastalarda profilaktik tedavi düşünülmelidir.

**Anahtar Kelimeler:** Koroner arter hastalığı, miyokard infarktüsü, PAI-1, MTHFR, Beta fibrinojen, Faktör XIII.



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## Introduction

Coronary artery disease is one of the main cardiovascular diseases caused by genetic and environmental factors and the interactions between them[1]. It is a major cause of death and disability in the world[2]. Myocardial infarction is a concept that refers to the damage of the heart muscles due to the reduction of blood flow to the heart as a result of the formation of plaques on the inner walls of the arteries and lack of oxygen[3]. Coronary artery disease, or coronary atherosclerosis, is a condition characterized by narrowing of the arteries that supply the heart muscle. Coronary angiography is the gold standard for the diagnosis of CAD and percutaneous coronary interventions[4]. Most cases of myocardial infarctions are caused by coronary artery disease. A heart attack is a life-threatening condition that occurs as a result of blockage in one or more coronary arteries and leads to serious tissue damage. The lack of oxygen in the myocardium for a certain period of time results in cell death and necrosis. The genetic basis of coronary artery disease is important for the formation and course of the disease. In this context, the effect of thrombophilic variants on coronary artery disease and myocardial infarction is controversial. For example, Park et al. argue that the 4G > 5G polymorphism located in the Plasminogen activator inhibitor-1 (PAI-1) promoter region is associated with coronary artery disease as well as other atherosclerotic diseases such as venous thromboembolism, ischemic stroke, carotid artery stenosis and renal artery stenosis[5]. The 4G/4G genotype and the 4G allele of the PAI-1 gene are thought to be associated with the risk and morbidity of acute myocardial infarction (AMI). The 4G/4G genotype of PAI-1 may also be associated with mortality from AMI[6]. The association of the PAI-1 4G/5G polymorphism with the plasma concentration of PAI-1 has also been reported in different studies[7]. Methylenetetrahydrofolate reductase (MTHFR) is an enzyme that plays an important role in the breakdown of homocysteine (Hcy), a by-product of cysteine metabolism. High homocysteine levels are associated with an increased risk of myocardial infarction. A clear association between the MTHFR C677T polymorphism and the risk of CAD has been demonstrated in the Chinese population[8]. It is also expressed that homozygous TT variant of MTHFR gene in Eastern Black Sea Turks is a risk factor for MI patients[9]. Rallidis et al. assert that the presence of the A allele for the G-455A polymorphism of the β-fibrinogen gene has a protective effect against the development of non-fatal acute MI in the Greek population aged ≤35 years[10]. The FXIII-V34L variant is claimed to reduce the risk of thrombosis[11]. It has also been suggested that the FXIIIA V34L polymorphism may be protective for MI in Caucasians[12]. Based on these discussions and similar considerations, the aim of this study was to analyze the relationship between coronary artery disease-myocardial infarction and MTHFR C677T, MTHFR A1298C, PAI-1 4G/5G, Beta fibrinogen G-455A, FXIII V34L gene polymorphisms.

## Material Method

### *Study Population*

The study included CAD patients diagnosed with electrocardiogram (ECG), exercise ECG and/or coronary angiography at Sivas Cumhuriyet University Research Hospital and heart attack cases treated in the emergency department of the same hospital between 2006 and 2022. In this research, 64 CAD patients, 12 of whom had MI were analyzed together with 64 healthy controls. This study was approved by the ethics committee of Sivas Cumhuriyet University (Decision no: 2023-07/01 Date: 20.07.2023).

### *Laboratory Analysis*

Genomic DNA was isolated using the EZ1 blood mini kit and polymerase chain reaction (PCR) was performed using the PyroMark PCR Kit (Qiagen, Germany). After 15 min activation at 95°C, The DNA was amplified for 45 cycles under the following conditions: 30 sec at 94°C for denaturation, 30 sec at 60°C for annealing and 30 sec at 72°C for extension. PCR was completed with 10 min final extension at 72°C. PCR products (10 µL) were mixed with streptavidin-conjugated sepharose material in binding buffer (70 µL). They were collected using a vacuum workstation and added to the sequencing primer and annealing buffer in Q24 plate and kept at 80°C for 2 minutes. Pyrosequencing reaction was performed through PyroMark Q24 instrument (Qiagen, Germany).

### *Statistical Analysis*

Statistical analysis was performed with SPSS 22.0 program (SPSS Inc., Chicago, IL, USA). Independent Samples t Test was used for the comparison of mean age. In this comparison, data were presented as mean ± SD. All genotypes and alleles frequencies of patients and controls was compared by x2 test. The odds ratios were calculated at 95% confidence interval and P value < 0.05 was accepted statistically significant.

## Results

Sixty-four CAD-MI patients and 64 healthy controls were evaluated for polymorphisms of MTHFR C677T, MTHFR A1298C, FXIII V34L, Beta fibrinogen G-455A and PAI-1 4G/5G. The age was higher in the patient group (P: 0.001). The mean age of the patients was 66.6 years and that of the control group was 49.8. The number of males was higher in the patient group than in the control group and there were much more smokers in the patient group. Twenty-eight of the patients also had hypertension. (Table 1). There was a statistically significant difference between the patient and control groups in terms of PAI-1 4G/5G genotype and 4G allele frequencies (Table 2). The 4G/4G genotype and 4G allele frequency were significantly higher among patients compared to the control group (P=0.01). Although mutant alleles for MTHFR C677T, MTHFR A1298C, Beta fibrinogen and FXIII variants were higher in the patient group, there was no statistically significant difference between the patients and control group (Table 3-5).

## Discussion

Prospectively, genetic testing may identify subgroups of patients at high risk of CAD for the therapeutic or prophylactic approach[13]. A number of gene polymorphisms affecting hemostasis have been identified, and among these, β-fibrinogen−455 G/A and PAI1 4G/5G gene polymorphisms have been associated with both myocardial infarction and CAD[14]. Plasminogen activator inhibitor-1 (PAI-1) is a serine protease inhibitor, that suppresses fibrinolysis by inhibiting both tissue-type and urokinase-type plasminogen activator[15]. It has been suggested that the 4G allele is more active than the 5G allele. In other words, PAI-1 concentrations tend to be higher in the presence of the 4G allele compared to the 5G allele. In this context, homozygous 4G/4G polymorphism has been associated with increased PAI-1 activity and increased risk of thrombosis[16]. Therefore, Zhang et al. suggested that PAI-1 4G/5G polymorphism may be a possible biomarker for the risk of venous thromboembolism[17]. In a study conducted in Brazil, it was found that PAI-1 was independently associated with CAD, and PAI-1 levels were higher in patients with the 4G/4G genotype[18]. In our study, PAI-1 4G allele and 4G/4G genotype were significantly associated with coronary artery disease and myocardial infarction (P:0.01). MTHFR is a major enzyme in homocysteine metabolism that catalyzes the reduction of 5-10-MTHF to 5-MTHF, a circulatory form of folate that is effective in the remethylation of homocysteine to methionine. MTHFR gene mutations affect Hcy level and may contribute to hyperhomocysteinemia, decreased folate levels, and various cardiovascular diseases[19]. While Shivkar et al. observed a statistically significant hyperhomocysteinemia in carriers of the T allele for MTHFR C677T genotype in the young coronary artery disease group, they could not establish such a link for the MTHFR A1298C polymorphism[20]. Friso et al. state that the 1298C allele is not associated with increased plasma homocysteine, regardless of folate status[21]. On the other hand, high Hcy levels with the T allele of MTHFR C677T polymorphism and the A allele of A1298C polymorphism has been linked with AMI and massive and sub massive pulmonary thromboembolism[22]. In a study by Husemoen et al., the risk of ischemic heart disease was higher in individuals with the MTHFR TT genotype, independent of folate status[23]. Although mutant alleles were more common in the patient group, no significant association was found between coronary artery disease and MTHFR C677T-A1298C polymorphisms in the current study. FXIII is a protein that catalyzes the formation of covalent cross-links between fibrin monomers to stabilize the clot[24]. FXIII-A V34L has been associated with a protective effect in relation to thrombotic disease. Some studies assert that FXIII-A V34L may be linked with a reduced risk of myocardial infarction and coronary artery disease[25]. Various factors are known to affect plasma fibrinogen concentrations. These include the β-fibrinogen -455G/A gene single nucleotide polymorphism, and the A allele in this SNP has been shown to be associated with high plasma fibrinogen levels[26]. In the study of Lu et al., the A allele frequency of the G-455A polymorphism of the β-fibrinogen gene was significantly lower in MI cases than controls, in Chinese Han population[27]. This suggests that the A allele may be protective for MI. Conversely, Chen et al. claim that the -455G/A polymorphism of β-fibrinogen gene may be associated with the propensity for coronary artery disease in China and the A allele of this polymorphism increases susceptibility to this disease[28]. No significant difference was observed between the patient group and the control group in terms of FXIII and beta fibrinogen gene variants in our study.

In conclusion, we were unable to establish an association between MTHFR C677T, MTHFR A1298C, β-fibrinogen -455G/A and FXIII V34L

variants with coronary artery disease and MI. However, PAI-1 4G/5G gene polymorphism was significantly associated with coronary artery disease. We believe that the 4G allele and 4G/4G genotype of this marker contribute to coronary artery disease and thus MI cases remarkably. It would be meaningful to consider prophylactic antithrombophilic treatment in cases with this variant in risk groups.

**Table 1. Characteristics of CAD-MI patients and controls**

| | Patients | Controls |
|---|---|---|
| **Age** | 66.6 ±12 | 49.8±11 |
| **Sex** | 49 M (76.6%) 15 F (23.4%) | 36 M (56.3%) 28 F (43.7%) |
| **Smoking** | 44 (68.8%) | 12(18.8%) |
| **Hypertension** | 28 (43.8%) | - |

**Table 2. The distribution of PAI-1 4G/5G genotypes and allele frequencies in CAD-MI cases and control groups**

| PAI-1 4G/5G Genotype&Allele | CAD-MI n:64 | Control n: 64 | P value |
|---|---|---|---|
| 5G/5G | 5 (7.8%) | 17 (26.6%) | |
| 4G/5G | 40 (62.5%) | 37 (57.8%) | P= 0.01 P<0.05 |
| 4G/4G | 19 (29.7%) | 10 (15.6%) | |
| 5G | 50 (39%) | 71 (55.5%) | P= 0.01 |
| 4G | 78 (61%) | 57 (44.5%) | P<0.05 |
| Odds ratio | 0.515 (0.31-0.85) %95 CI | | |

**Table 3. The distribution of MTHFR C677T genotypes and allele frequencies in CAD-MI cases and control groups.**

| MTHFR C677T Genotype&Allele | CAD-MI n:64 | Control n: 64 | P value |
|---|---|---|---|
| CC | 30 (7.8%) | 32 (26.6%) | |
| CT | 27 (62.5%) | 27 (57.8%) | P= 0.82 P>0.05 |
| TT | 7 (29.7%) | 5 (15.6%) | |
| C | 87 (39%) | 91 (55.5%) | P= 0.59 |
| T | 41 (61%) | 37 (44.5%) | P>0.05 |
| Odds ratio | 0.863 (0.51-1.47) %95 CI | | |

**Table 4. The distribution of MTHFR A1298C genotypes and allele frequencies in CAD-MI cases and control groups.**

| MTHFR A1298C Genotype&Allele | CAD-MI n:64 | Control n: 64 | P value |
|---|---|---|---|
| AA | 22 (34.4%) | 27 (42.2%) | |
| AC | 31 (48.4%) | 29 (45.3%) | P= 0.59 |
| CC | 11 (17.2%) | 8 (12.5%) | P>0.05 |
| A | 75 (39%) | 83 (55.5%) | P= 0.30 |
| C | 53 (61%) | 45(44.5%) | P>0.05 |
| Odds ratio | | 0.767 (0.46-1.27) %95 CI | |

**Table 5. Distribution of Beta-fibrinojen -455 G>A & FXIII allele frequencies in CAD-MI cases and control groups.**

| Beta-fibrinojen -455 G>A | CAD-MI n:64 | Control n: 64 | P value |
|---|---|---|---|
| G | 101 (78.9%) | 103 (80.5%) | P= 0.75 |
| A | 27 (21.1%) | 25 (19.5%) | P>0.05 |
| Odds ratio | | 0.908 (0.49-1.67) %95 CI | |
| FXIIIV34L | MI/KAH n:64 | Control n: 64 | P value |
| V | 99 (77.3%) | 103 (80.5%) | P= 0.54 |
| L | 29 (22.7%) | 25 (19.5%) | P>0.05 |
| Odds ratio | | 0.829 (0.45-1.51) %95 CI | |

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