

# COMPARISON OF GENE POLYMORPHISMS IN SEVERE ATHEROSCLEROTIC PATIENTS IN DIFFERENT AGE GROUPS

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## ABSTRACT:

**Purpose:** To evaluate the association of genetic polymorphisms of angiotensin converting enzyme, factor V, endothelial nitric oxide synthase, and interleukin-6 in a group of patients that have coronary artery disease.

**Materials and Methods:** Patients were assigned into three groups of 20, one being the control and the other two being the study groups 1 and 2. Group 1 consisted of patients less than 40 years of age with severe coronary artery disease. Group 2 consisted of patients older than 70 years of age with severe coronary artery disease. The control group consisted of patients over 70 years of age with normal coronary arteries. Allele frequencies for angiotensin converting enzyme, factor V, endothelial nitric oxide synthase, and interleukin-6 gene polymorphisms were compared.

**Results:** The allele frequencies for angiotensin converting enzyme, endothelial nitric oxide synthase, and interleukin-6 in groups 1 and 2 were not found to be significantly different from those of the control group. Factor V Leiden 'A' allele frequency was found to be significantly higher in group 1 when compared with the control.

**Conclusion:** In the present study angiotensin converting enzyme, endothelial nitric oxide synthase, and interleukin-6 gene polymorphisms were not associated with coronary artery disease in young or elderly patients. However, in the younger group, Factor V Leiden 'A' allele frequency was significantly higher than that in the control group. Thus, prothrombotic risk factors might be considered an etiological factor for coronary artery disease in younger patients.

**Key words:** Genetic Polymorphism, Angiotensin Converting Enzyme, Endothelial Nitric Oxide Synthase, Interleukin-6, Factor V

## CİDDİ ATEROSKLEROZU BULUNAN FARKLI YAS GRUPLARINA AİT HASTALARDA GÖRÜLEN GEN POLİMORFİZMLERİNİN KARSILASTIRILMASI

### ÖZ:

**Amaç:** Bu çalışma, koroner arter hastalığı bulunan bir grup hastada anjiyotensin dönüştürücü enzim, faktör V, endotelial nitrik oksit sentaz ve interleukin-6 genetik polimorfizmlerinin birlikteliğini değerlendirmektedir.

**Gereç ve Yöntem:** Hastalar, biri kontrol ve diğer ikisi çalışma grubu 1 ve çalışma grubu 2 olarak, 20'er kişiden oluşan 3 gruba ayrıldılar. Grup 1'e ait hastalar, 40 yaşın altında ve ciddi koroner arter hastalığı bulunan hastalardan oluştu. Grup 2'ye ait hastalar, 70 yaşın üstünde ve ciddi koroner arter hastalığı olan kişilerden oluştu. Kontrol grubundaki hastalar, 70 yaşın üzerinde ve normal koroner arterlere sahip olan kişilerden oluştu. Gruplar arasında anjiyotensin dönüştürücü enzim, faktör V, endotelial nitrik oksit sentaz ve interleukin-6 gen polimorfizmlerine ait allel sıklıkları karşılaştırıldı.

**Bulgular:** Grup 1 ve Grup 2'deki hastalarda tespit edilen, anjiyotensin dönüştürücü enzim, faktör V, endotelial nitrik oksit sentaz ve interleukin-6 gen polimorfizmlerine ait allel sıklıkları kontrol grubundaki deneklerden elde edilen allel sıklıklarından anlamlı derecede farklı bulunmadı. Faktör V Leiden "A" allel sıklığı, Grup 1'e ait hastalarda, kontrol grubuna ait hastalardan anlamlı olarak daha yüksek sıklıkta bulundu.

**Sonuç:** Bu çalışmada gerek genç gerekse yaşlı kişilerde, anjiyotensin dönüştürücü enzim, faktör V, endotelial nitrik oksit sentaz ve interleukin-6 genetik polimorfizmleri, koroner arter hastalığı ile bağlantılı bulunmadı. Ancak genç hastalarda, faktör V Leiden "A" allel sıklığı kontrol grubuna ait hastalardan anlamlı olarak daha yüksekti. Bu nedenle, gençlerde görülen koroner arter hastalıklarında protrombotik risk faktörlerinin etyolojide rol aldıkları düşünülebilir.

**Anahtar Kelimeler:** Genetik Polimorfizm, Anjiyotensin Dönüştürücü Enzim, Endotelial Nitrik Oksit Sentaz Ve İnterlökin-6, Faktör V

## INTRODUCTION

Coronary artery disease (CAD) is the result of the development of atherosclerosis in coronary arteries and is a major cause of mortality and morbidity. Positive family history, lipid metabolism defects, diabetes mellitus, hypertension, smoking, and obesity are important risk factors for CAD.<sup>1</sup> Persistent dysfunction of the endothelium in affected arteries is an important aspect of atherosclerosis.<sup>2</sup> Inflammatory processes are also known to play a role in atherogenesis.<sup>3</sup> Apart from environmental factors, intrinsic impairment of the expression of endothelial gene products involved in the maintenance of vascular homeostasis may predispose to atherosclerosis.<sup>2</sup>

The genetic basis of the pathogenesis of atherosclerosis is under investigation. The frequency of CAD is higher in monozygotic twins than in dizygotic twins.<sup>4</sup> This constitutes evidence for the role of genetic factors in the pathogenesis of CAD. Decryption of the characterizing genetic polymorphisms may help to define genetic risk factors for CAD.

This prospective case-control study evaluates the association of genetic polymorphisms of angiotensin converting enzyme (ACE), factor V (FV), endothelial nitric oxide synthase (e-NOS), and interleukin-6 (IL-6), which are thought to be potentially related to CAD. The presence of these polymorphisms in young (<40 years) and elderly (>70 years) CAD patients who underwent coronary artery bypass grafting (CABG) were compared with the polymorphisms in a control group proved to have normal coronary arteries by coronary angiographic studies.

## MATERIALS AND METHODS

This prospective study was conducted between November 2006 and March 2008. The approval of the local ethical committee and informed consent from the patients were obtained.

Patients were assigned into three groups. Group 1 consisted of 20 patients less than 40 years of age who underwent CABG for severe CAD. Group 2 consisted of 20 patients older than 70 years of age who underwent CABG for severe CAD. The control group consisted of 20 patients over 70 years of age whose coronary arteries were shown to be normal by coronary angiography. Only one control group was used because genetic material does not change with age. Genetic mutations of ACE, FV, e-NOS, and IL-6 were studied and compared with those in the control group.

Genomic DNA was extracted from peripheral blood leucocytes (200 µl of total blood) by using a Macherey-Nagel (MN) Nucleospin blood® DNA extraction kit (cat. no. 740.951.250) according to the manufacturer's instructions.

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The insertion/deletion polymorphism of a 287 bp sequence within the intron 16 of the ACE gene and restriction fragment length polymorphism (RFLP) from intron 4 of the e-NOS gene were analyzed by the methods previously described.<sup>5,6</sup>

The missense Leiden mutation in Factor V gene causes the change of aminoacid Arg506Gln. Real time PCR technology was employed to analyze the mutation G1691A according to the manufacturer's instruction (Dr. Zeydanli Life Science Tech. cat no. DZFBV).

A previously published method was used to detect the IL-6-174 G/C variant in patients.<sup>7</sup>

### Statistical Analysis

The Statistical Package for the Social Sciences (SPSS), v.13.0 for Windows, was used to analyze the data. The distribution of ACE, eNOS, FV, and IL-6 gene polymorphisms were compared among the groups using the X<sup>2</sup> test. A p value <0.05 was considered statistically significant.

### RESULTS

Patients' characteristics are shown in Table 1. Group 1 was found to be significantly younger and also had significantly frequent positive family history for CAD when compared with the control group. Sex, presence of hypertension, hypercholesterolemia, diabetes mellitus, and smoking did not differ among the groups (Table 1).

**Table 1.** Patients' characteristics.

	Group 1	Group 2	Control group
Age	37.5±6.4 †	71.2±4.2	70.7±6.9
Sex (Female)	2 (10.0%)	2 (10.0%)	3 (15.0%)
Hypertension	9 (45.0%)	8 (40.0%)	13 (65.0%)
Hypercholesterolemia	7 (35.0%)	8 (40.0%)	6 (30.0%)
Diabetes mellitus	2 (10.0%)	4 (20.0%)	5 (25.0%)
Smoking	12 (60.0%)	5 (25.0%)	6 (30.0%)
Family history	9 (45.0%) †	4 (20.0%)	2 (10.0%)

† : p<0.0

The distribution of "DD", "ID", and "II" genotypes of the ACE gene was 9 (45.0%), 9 (45.0%), and 2 (10.0%) in group 1; 7 (35.0%), 12 (60.0%), and 1 (5.0%) in group 2; and 6 (30.0%), 9 (45.0%), and 5 (25.0%) in the control group. The allele frequencies of ACE gene polymorphism were calculated. The frequencies of 'D' and 'I' allele in groups 1 and 2 were not significantly different from those in the control group (p=0.2, p=0.1) (Table 2).

**Table 2.** Allele frequencies of different gene polymorphisms.

	Group 1	Group 2	Control group
ACE polymorphism			
<i>D</i> allele	27(67.5%)	26(65.0%)	21(52.5%)
<i>I</i> allele	13(32.5%)	14(35.0%)	19(47.5%)
eNOS polymorphism			
<i>a</i> allele	4(10.0%)	7(17.5%)	8(20.0%)
<i>b</i> allele	36(90.0%)	33(82.5%)	32(80.0%)
FV polymorphism			
<i>G</i> allele	28(70.0%)	31(77.5%)	37(92.5%)
<i>A</i> allele	12(30.0%) †	9(22.5%)	3(7.5%)
IL-6 polymorphism			
<i>C</i> allele	6(15.0%)	11(27.5%)	12(30.0%)
<i>G</i> allele	34(85.0%)	29(72.5%)	28(70.0%)

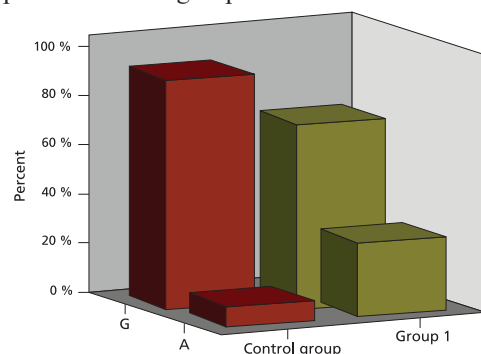
† : p<0.05

The "BB", "BA", and "AA" genotype frequencies of the VNTR polymorphism in intron 4 of the e-NOS gene were 16 (80.0%), 4 (20.0%), and 0 (0%) in group 1; 14(70.0%), 5(25.0%), and 1 (5.0%) in group 2; and 13 (65.0%), 6 (30.0%), and 1 (5.0%) in the control group. The frequencies of 'B' and 'A' allele in groups 1 and 2 were not significantly different from those in the control group (p=0.3, p=1) (Table 2).

The "CC", "GC", and "GG" genotype frequencies of the IL-6 gene were 0 (0%), 6 (30%), and 14 (70%) in group 1; 1 (5.0%), 9 (45.0%), and 10 (50.0%) in group 2; and 1 (5.0%), 10 (50.0%), and 9 (45.0%) in the control group. The frequencies of 'C' and 'G' allele in groups 1 and 2 were not significantly different from those in the control group (p=0.1, p=1) (Table 2).

The "GG", "GA", and "AA" genotype frequencies of the FV gene were 11 (55.0%), 6 (30.0%), and 3 (15.0%) in group 1; 12 (60.0%), 7 (35.0%), and 1 (5.0%) in group 2; and 17 (85.0%), 3 (15.0%), and 0 (0%) in the control group. 'A' allele frequency was significantly higher in group 1 when compared with the control group (p=0.02, p=0.1) (Table 2) (Figure 1).

**Figure 1.** FV Leiden 'G' and 'A' allele frequency in Group 1 and Control group



## DISCUSSION

Knowing the risk factors for CAD, which constitutes the number one cause of mortality in developed countries, is of utmost importance for disease prevention. Although there are plenty of studies evaluating multiple environmental factors causing CAD, the scientific literature offers few studies evaluating the genetic basis for the disease. In this study, four types of genetic polymorphisms potentially responsible for the development of CAD in young and elderly CAD patients were compared to those in a control group.

Vascular ACE is a membrane-bound enzyme localized on endothelial cells and it is also present in the smooth muscle cells and adventitial layers of the blood vessels.<sup>8</sup> The direct cellular effect of angiotensin plays a role in cell proliferation. It has been reported that the D allele of the ACE gene is associated with increased activity of tissue and serum ACE. Angiotensin converting enzyme levels were found to be lowest in individuals who were homozygous for the I allele.<sup>9</sup> Bautista et al. showed that the ACE DD genotype carried a 1.98 times higher risk of MI when compared with the combined group of genotypes II and ID.<sup>10</sup> Ciećwierz stated that the DD genotype is a marker of impaired left ventricular function in relatively young patients with coronary heart disease.<sup>11</sup> Völzke et al. reported that the ACE DD genotype is associated with increased midterm mortality and cardiac mortality after CABG. These relationships have been attributed to increased ACE II activities in those who have the ACE D allele.<sup>12</sup> In contrast, Tutun et al. have found similar allele frequencies in a group of young CAD patients when compared to a control group.<sup>13</sup> Hubacek et al. reported that I/D polymorphism in the gene for ACE is not a genetic risk factor for myocardial infarction.<sup>14</sup> In our study although D allele frequency in group 1 was higher than that in the control group, the difference was not statistically significant.

Endothelial nitric oxide synthase (eNOS) is expressed in the endothelium and produces NO from L-arginine. NO inhibits the smooth muscle cell proliferation, platelet aggregation, and leukocyte adhesion.<sup>15, 16</sup> Furthermore, NO contributes to the basal vascular tone and regulates blood flow.<sup>17</sup> If these processes are altered by reduced eNOS activity, the risk of vasospasm and thrombosis may increase, which may cause atherosclerosis. Some studies have shown possible associations between eNOS gene polymorphisms and atherosclerosis. Wang et al. reported that the eNOS 4a/b genotype was related to the severity of atherosclerosis.<sup>18</sup> Heltianu et al. suggested that eNOS 4b/a confers a relatively high risk of hypertension in subjects with atherogenic risk factors.<sup>19</sup> In contrast, Hibi et al. did not find any evidence of accelerated atherosclerosis among patients with 'aa' genotype of eNOS 4a/b polymorphism.<sup>20</sup> In the present study, we also did not find any significant differences between the study and control groups regarding 'a' and 'b' allele frequencies.

Interleukin-6 (IL-6) has an important role in inflammatory processes that stimulate endothelial activation, vascular smooth muscle cell proliferation, and leukocyte

activation.<sup>20,21</sup> These effects may accelerate atherosclerosis by giving way to plaque growth or instabilization.<sup>20, 22</sup> Ridker et al. demonstrated an association between elevated IL-6 levels and the risk of myocardial infarction.<sup>21</sup> Genetic studies are carried out to explain this issue. Brull et al. showed that elevated IL-6 levels were found in patients with -174G>C polymorphism after CABG.<sup>20</sup> Tutun et al. studied -174G>C polymorphism in young patients who underwent CABG. They showed a statistically significant difference between groups for 'CC' homozygosity in young patients who underwent CABG for CAD.<sup>13</sup> Gaudino et al. identified the 'GG' genotype as a predictor of postoperative atrial fibrillation via elevated IL-6 levels.<sup>23</sup> We did not find any significant differences between groups 1 and 2 and the control group regarding -174G>C polymorphism. The frequency of the 'GG' genotype was higher in group 1 than in the control group (45% vs. 70%) but the difference was not statistically significant ( $p=0.1$ ).

Factor V (FV) Leiden is known as a mutagenic factor and it is resistant to activated protein C. Factor V Leiden is inactivated 10 times slower than normal FV.<sup>24</sup> The prevalence of FV Leiden frequency is reported to be 7.1% in a Turkish population.<sup>25</sup> Patients who have 'AA' homozygosity were found to have a higher risk of thrombosis.<sup>26, 27</sup> Although FV Leiden is known to be a common risk factor for venous thrombosis,<sup>28</sup> its relation to CAD is still a subject of controversy. Vallus et al. reported that FV (Leiden) mutation may influence the progression of atherosclerosis and the development of restenosis after revascularization in patients with accelerated femoropopliteal atherosclerosis.<sup>29</sup> Tutun et al. reported that 'GA' heterozygous mutation was significantly higher in young patients who underwent CABG than in control patients.<sup>13</sup> Furthermore, Rosendaal et al. found a significant association between FV Leiden mutation and CAD in young women.<sup>30</sup> The tendency for thrombosis in the patients included in the above studies was thought to be the reason for myocardial infarction. However, several other studies found that FV Leiden is not a risk factor for CAD.<sup>31, 32</sup> In our study the 'A' allele was significantly higher in group 1 than the control group ( $p=0.02$ ). In addition, we did not find any significant differences between group 2 and the control group regarding 'A' and 'G' allele frequency ( $p=0.1$ ).

## CONCLUSIONS

In our study, ACE, eNOS, and IL-6 gene polymorphisms were not found to be associated with CAD in young or elderly patients compared with the control group. However, in the younger group, factor V Leiden 'A' allele frequency was significantly higher than it was in the control group. This discrepancy was not observed between group 2 and the control group. Thus, prothrombotic risk factors might be considered for CAD in younger patients. Although the sample size of the present study is relatively small, its prospective design and comparison of younger and older patients groups constitute a significant distinction. This study may illuminate the role of the genetic factors for the development of CAD in younger patients.

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