

# The Distribution of Apolipoprotein E Gene Polymorphism and Apolipoprotein E Levels among Coronary Artery Patients Compared to Controls

## Kontroller ile Karşılaştırıldığında Koroner Arter Hastalarında Apolipoprotein E Gen Polimorfizmi ve Apolipoprotein E Düzeyleri Arasındaki Dağılım

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### Abstract

**Objective:** Coronary artery disease (CAD) is a multifactorial disease that is caused by various genetics and environmental factors. Genetically, predisposition is an important component for CAD. The candidate apolipoprotein E (apoE) gene is the most studied one. ApoE is composed of e2, e3, e4 alleles and E2/2, E2/3, E2/4, E3/3, E3/4, E4/4 genotypes. In this study, the relationship between CAD and apoE polymorphism and apoE level has been studied in Tokat region.

**Materials and Methods:** The study population is composed of 100 CAD patients diagnosed by coronary angiography and 100 control patients of whom fifty have normal coronary angiography and fifty did not have any CAD symptoms. The serum lipid and apoE levels and apoE genotypes of all participants have been measured, and the relationship between these parameters has been evaluated.

**Results:** Apolipoprotein E, total cholesterol, HDL cholesterol and LDL cholesterol levels were statistically low at CAD patients than control patients ( $p=0.0004$ ,  $p=0.0005$ ,  $p=0.0107$ ,  $p=0.0052$  respectively). There was not any significant difference between triglyceride levels ( $p=0.0848$ ). Waist circumferences were significantly high at CAD patients ( $p=0.0012$ ). Allele frequencies were as e2 (7.25%), e3 (83.5%), e4 (9.25%) and genotype distributions were as E2/2 (0.5%), E2/3 (13%), E2/4 (0.5%), E3/3 (68.5%), E3/4 (16.5%), E4/4 (1%). The distribution of alleles and genotypes were not significantly different ( $p>0.05$ ). ApoE levels were higher at e2 allele carriers than e3 and e4 allele carriers ( $p<0.05$ ). However, there was no significant difference between e3 and e4 allele carriers.

**Conclusion:** In conclusion, the distribution of apoE genotype and allele at our region is similar to the general of Turkey. The low apoE levels in CAD patients may show the influence of apoE on CAD by local and systemic mechanisms.

**Keywords:** Coronary artery disease, apoE genotype, apoE level

### Öz

**Amaç:** Koroner arter hastalığı (KAH), çeşitli genetik ve çevresel faktörlerin neden olduğu multifaktöriyel bir hastalıktır. KAH riskinin önemli bir bileşeni genetik yatkınlıktır. Aday genlerden apolipoprotein E (APOE) en fazla çalışılanıdır. APOE;  $\epsilon 2$ ,  $\epsilon 3$ ,  $\epsilon 4$  allelleri ve APOE2/2, APOE2/3, APOE2/4, APOE3/3, APOE3/4, APOE4/4 genotiplerinden oluşmaktadır. Bu çalışmada; Tokat bölgesindeki koroner arter hastalarında APOE gen polimorfizmi ve APOE düzeyi arasındaki ilişki araştırılmıştır.

**Gereç ve Yöntem:** Çalışmaya kardiyak kateterizasyon ile KAH tanısı konulan 100 hasta ve 100 kişilik kontrol grubu dahil edilmiştir. Tüm bireylerin serum lipid, APOE düzeyi ve APOE genotipleri belirlenmiş ve bunlar arasındaki ilişki araştırılmıştır. ApoE seviyeleri nefelometrik olarak, APOE gen polimorfizmi ise hibridizasyon prob metodu ile tayin edilmiştir.

**Bulgular:** İstatistiksel olarak KAH'na sahip bireylerde APOE, total kolesterol, HDL kolesterol, LDL kolesterol düzeyleri kontrol grubuna göre daha düşüktü (sırasıyla;  $p=0,0004$ ,  $p=0,0005$ ,  $p=0,0107$ ,  $p=0,0052$ ). Trigliserid düzeylerinde anlamlı bir farklılık yoktu ( $p=0,0848$ ). Bel çevreleri koroner arter hastalarında anlamlı olarak yüksekti ( $p=0,0012$ ). Allel frekansları  $\epsilon 2$  (%7,25),  $\epsilon 3$  (%83,50),  $\epsilon 4$  (%9,25) ve genotip dağılımları APOE2/2 (%0,5), APOE2/3 (%13), APOE2/4 (%0,5), APOE3/3 (%68,5), APOE3/4 (%16,5), APOE4/4 (%1) şeklindeydi. Allel ve genotiplerin dağılımlarında, gruplar arasında anlamlı bir farklılık bulunamadı ( $p>0,05$ ). APOE2 alleli taşıyanlarda  $\epsilon 3$  ve  $\epsilon 4$  allellere göre APOE düzeyi anlamlı olarak yüksekti ( $p<0,05$ ),  $\epsilon 3$  ve  $\epsilon 4$  allelleri arasında ise farklılık gözlenmedi.

**Sonuç:** Sonuç olarak bölgemizde APOE genotip ve allel dağılımı Türkiye geneli ile benzer dağılım göstermektedir. APOE düzeylerinin koroner arter hastalarında düşük bulunması APOE'nin lokal veya sistemik mekanizmalarla KAH'nın etiyopatogenezinde etkili olabileceğini düşündürmektedir.

**Anahtar Kelimeler:** Koroner arter hastalığı, APOE genotipi, APOE düzeyi

This study was presented at the 10<sup>th</sup> National Congress of Medical Genetics in December 2012, Bursa, Turkey.

**Received:** February 11, 2015 / **Accepted:** June 04, 2015

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DOI: 10.5152/eurasianjmed.2015.25



## Introduction

Coronary artery disease (CAD) is a multifactorial disease caused by various genetic and environmental factors [1]. Studies conducted over CAD revealed a direct association between gene variants and disease manifestation [2-4]. Among the candidate genes, apolipoprotein E (*APOE*) gene is the one subjected to the highest number of studies [5]. *APOE* is found in blood typically in 0.02-0.06 g/L concentrations along with chylomicrons, very low-density lipoprotein (VLDL) and high-density lipoprotein (HDL). It is synthesized in liver, macrophages and many other tissues [6, 7]. *APOE* has three common alleles, i.e.  $\epsilon 2$ ,  $\epsilon 3$  and  $\epsilon 4$ , and six (*APOE2/2*, *APOE2/3*, *APOE2/4*, *APOE3/3*, *APOE3/4*, *APOE4/4*) genotypes [8]. The gene encoding these three isoforms is located on the human chromosome 19 long arm (19q13.2) and is closely linked with *APOC-I/C-II* complex [9]. It has been reported that *APOE* genotype explains 7% of the variation for total cholesterol in healthy white population [10]. In Caucasians,  $\epsilon 4$  variant has 15% allele frequency and is associated with early atherosclerosis, mortality, stroke, Alzheimer's disease and myocardial infarction [7, 11, 12]. Low-density lipoprotein (LDL) cholesterol concentrations in  $\epsilon 4$  carriers are 8.3% higher than in  $\epsilon 3$  homozygous individuals. LDL cholesterol concentrations in  $\epsilon 2$  carriers, on the other hand, are 14.2% lower than in  $\epsilon 3$  homozygous individuals [13-15]. It has been shown by different studies that individuals carrying  $\epsilon 2$  has 40% higher CAD death risk than individuals with *APOE3/3* genotype or individuals carrying  $\epsilon 2$  [16].

The aim of the present study was to investigate the association between CAD and *APOE* gene polymorphism and *APOE* level in Tokat province of Turkey.

## Materials and Methods

This study included 100 CAD patients and 100 healthy control subjects. CAD group consisted of patients who applied to cardiology out-patient clinic of Gaziosmanpasa University, Health Research and Application Center. CAD was identified by cardiac catheterization as having >50% stenosis of one major coronary artery. Control group consisted of 50 people whose cardiac catheterization showed stenosis <10% of one major coronary artery and 50 healthy subjects without any clinical signs of CAD. These healthy subjects were clinically and laboratorially (electrocardiography, chest x-ray, effort test) analysed before the study. Approval of the university's ethic committee was taken before the study. A total of 10 mL venous blood sample was taken from each patient in tubes with and without ethylene diamine tetra acetic acid (EDTA). Blood samples were centrifuged on 1500 x g for 15 minutes and isolated serum was stored at -80 °C. Lipid levels were then measured spectrophotometrically on thawed samples using Roche/Hitachi Modular P800 routine auto-analyser apparatus and commercial kit (Roche, Hitachi, Japan). *APOE* levels were determined nephelometrically using Dade Behring kit in Dade Behring BN II apparatus. For the determination of *APOE* gene polymorphism, deoxyribonucleic acid (DNA) isolation was carried out with whole blood using BioFlux kit (MagaZorb, Madison, US); *APOE* gene polymorphism was determined using a commercial kit (TIB MOLBIOL LightMix®Kit *APOE* C112R R158C) in Roche Light Cyclor 1.5 apparatus and hybridization probe method.

### Statistical analysis

One sample Kolmogorov-Smirnov test was employed to test the normal distribution of the data. For pair-wise com-

**Table 1. Characteristics of the people in the control group and coronary artery disease**

	CAD (n=100)	Control (n=100)	p
Sex (F/M)	50/50	50/50	
<i>APOE</i> (g/L)	0.0428±0.01	0.0479±0.01	0.0004
T. Cholesterol (mg/dL)	177.65±39.01	196.78±36.86	0.0005
Tryglyceride/mg/dL)	156.47±7.45	158.82±11.34	0.0848
HDL (mg/dL)	39.1±10.95	43.06±10.77	0.0107
LDL (mg/dL)	108.9±32.70	121.98±32.81	0.0052
Age (years)	59.68±10.16	49.66±11.44	<0.0001
Waist circumference (cm)	101.57±11.97	96±11.96	0.0012
Body mass index (kg/m <sup>2</sup> )	29.617±5.49	29.566±5.18	NS
T. Cholesterol: Total Cholesterol; HDL: High-Density Lipoprotein; LDL: Low-Density Lipoprotein; CAD: Coronary artery disease; NS: Not significant; <i>APOE</i> : Apolipoprotein E			

**Table 2. APOE genotype distribution in CAD and control groups**

	CAD	Control
APOE polymorphism		
APOE2/2	0 (0%)	1 (1%)
APOE2/3	12 (12%)	14 (14%)
APOE2/4	1 (1%)	0 (0%)
APOE3/3	73 (73%)	64 (64%)
APOE3/4	12 (12%)	21 (21%)
APOE4/4	2 (2%)	0 (0%)

CAD: Coronary artery disease; APOE: Apolipoprotein E

**Table 3. Distribution of APOE alleles of persons in CAD and control groups**

	CAD	Control
Allele frequency		
ε2	6.5%	8%
ε3	85.5%	81.5%
ε4	8%	10.5%

CAD: Coronary artery disease

**Table 4. Serum APOE levels according to APOE allele of patients in CAD and control groups**

	ε2	ε3	ε4
	APOE level (g/L)	APOE level (g/L)	APOE level (g/L)
CAD	0.0518±0.0064	0.0418±0.0109	0.0404±0.0141
Control	0.0599±0.0174	0.0457±0.0102	0.0461±0.0163
Total	0.0563±0.0140	0.0436±0.0107	0.0438±0.0155

CAD: Coronary artery disease; APOE: Apolipoprotein E

parisons, Student's t-test was used for normally distributed variables, while Mann-Whitney U test was used for the variables which were not normally distributed. For the evaluation of multiple groups, one-way variance analysis was used. For the analysis of categorical data, Chi-square and logistic regression were used. Pearson Correlation analysis was used to determine the associations between continuous variables. A probability level of  $p < 0.05$  was considered significant. SPSS (IBM; Chicago, IL, USA) v.10 was used for statistical analyses.

## Results

Characteristic features of patients and healthy subjects, who were given the extended form, in the study, were shown in Table 1. In CAD group, APOE level was significantly lower than

control group ( $p = 0.0004$ ). Compared to the control group, APOE, total cholesterol, HDL cholesterol, and LDL cholesterol levels were significantly lower in CAD group ( $p = 0.0004$ ,  $p = 0.0005$ ,  $p = 0.0107$  and  $p = 0.0052$ , respectively). There was no difference between the groups for triglyceride levels ( $p = 0.0848$ ). Average waist circumference was significantly higher in CAD group ( $p = 0.0012$ ). There were no statistical differences between groups for genotype and allele distributions (Table 2). APOE allele distribution did not vary significantly between study groups (Table 3). In terms of APOE levels of different alleles, ε2 allele had significantly higher APOE levels compared to ε3 and ε4 alleles across all study groups ( $p < 0.05$ ). There was no difference between ε3 and ε4 (Table 4). APOE levels had significant correlations with cholesterol, waist circumference and body mass index (BMI) in CAD patients group ( $p < 0.01$ ,  $p < 0.05$ ,  $p < 0.01$ , respectively).

**Limitations:** Significantly lower APOE levels observed in coronary artery disease patients may indicate that APOE may affect the etiopathogenesis of CAD via local or systemic mechanisms. Effects of APOE genotypes and alleles in etiopathogenesis of coronary artery disease need to be revealed in more detailed studies on their effects in mechanisms other than lipoprotein mechanism.

## Discussion

In addition to some risk factors, genetic factors are also known to be involved in the formation of coronary artery disease. There are many genes associated with CAD process and contribution of each gene to the process may be different. The most commonly studied gene variant related to CAD is APOE genotype [17]. In risk assessment studies, APOE3/3 genotype is taken as reference. In general, individuals carrying ε4 allele are considered to have cardiovascular disease risk [16], while ε2 allele is considered protective [17] or neutral for cardiovascular disease risk [11]. A study conducted among middle age men from nine populations showed that individuals carrying ε4 have 40% higher risk for CAD deaths compared to individuals carrying APOE3/3 or ε2 [16]. In some studies, individuals carrying ε4 have been found to be more prone to develop common coronary lesions and to have higher CAD mortality risk [11, 18]. In a study carried out on 199 subjects in Adana province of Turkey, total and LDL cholesterol levels were significantly higher and HDL cholesterol level was significantly lower in CAD group compared to healthy control subjects. Differences in triglyceride levels were not statistically significant [19]. In the present study, total, HDL and LDL cholesterol levels were significantly lower in CAD group than in the healthy control group. There was no difference between the groups for triglyceride levels. The reasons for lower values compared to control group could be due to

the differences in diets of the patients and medications used. HDL measurements more than 50 mg/dL in females and more than 40 mg/dL in males are correlated with a decline in risk of cardiovascular disease [20]. High HDL levels were found in terms of lower mortality risk to be significantly lower than the LDL level (<100 mg/dL) in the Japanese population [21]. In the present study, HDL cholesterol levels were significantly lower in CAD patient group and this finding is consistent with the literature [19, 21]. Compared to  $\epsilon 3$  allele,  $\epsilon 2$  allele was significantly associated with low LDL cholesterol levels. On the other hand,  $\epsilon 4$  allele was associated with higher LDL cholesterol levels [22]. Data from the Turkish Heart Study revealed that  $\epsilon 2$  alleles were associated with lower total and LDL cholesterol [8]. Considering the lipid levels and allele distributions, total and LDL cholesterol levels of  $\epsilon 2$  allele in CAD and control group were lower than those of  $\epsilon 3$  and  $\epsilon 4$  alleles, but the differences were not significant. Low values observed in the present study were consistent with the studies conducted on Turkish population as well as others in the literature [8, 22]. There was no difference between  $\epsilon 3$  and  $\epsilon 4$  alleles. As the average of all study groups,  $\epsilon 4$  allele had higher, though not significant, total and LDL cholesterol values.

*Apolipoprotein E* allele distribution shows great variations across the world. *APOE3* is considered the normal allele and has the highest frequency [23]. In the Turkish Heart Study, involving *APOE* allele and genotypes conducted on 8366 individuals, frequencies of  $\epsilon 3$ ,  $\epsilon 4$  and  $\epsilon 2$  alleles were determined to be 86.0, 7.9 and 6.1%, respectively [9]. In this study, frequencies of  $\epsilon 3$ ,  $\epsilon 4$  and  $\epsilon 2$  alleles in CAD patients were 85.5, 8.0 and 6.5%, respectively. Frequencies of  $\epsilon 3$ ,  $\epsilon 4$  and  $\epsilon 2$  in control group, on the other hand, were 81.5, 10.5 and 8%, respectively.

Apolipoprotein E gene polymorphism strongly affects the level of gene product [5]. Previous studies revealed that  $\epsilon 2$  allele is associated with higher levels of APOE, whereas  $\epsilon 4$  allele is associated with lower APOE levels [5, 24, 25]. APOE levels in CAD group were statistically lower than in healthy control group in the present study. APOE levels were highest in  $\epsilon 2$  and lowest in  $\epsilon 4$  alleles in all study groups, but the difference was more pronounced in CAD group. There was no difference between  $\epsilon 3$  and  $\epsilon 4$ . APOE levels were significantly higher in women in CAD group, but there was no difference between men and women in control group.

Obesity is associated with an increase in cardiovascular risk and mortality from all causes [26]. BMI is used as a measurement of obesity. Effect of abdominal obesity, measured by waist circumference, to increase cardiovascular risk is more pronounced. In the present study, APOE levels were correlated with the total cholesterol level, BMI and waist circumference values in CAD group. In CAD group, waist circumference was statistically higher than in control group. No difference was

observed for BMI between two groups. When BMI and waist circumference of men and women were evaluated, women had significantly higher BMI in the control group, and significantly higher BMI and waist circumference in CAD group.

In conclusion, distributions of *APOE* genotypes and alleles in our region are generally similar to those in Turkey. Considering the general information and our results with *APOE4* levels, it could be stated that  $\epsilon 4$  allele could be an important indication for evaluating the cardiovascular disease risk.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the ethics committee of Gaziosmanpaşa University School of Medicine.

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

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept - O.A.; Design - O.A., S.S.; Supervision - S.S.; Resources - S.S., H.O., K.C., O.A.; Materials - O.A., A.A., I.B.; Data Collection and/or Processing - O.A., A.A., K.C.; Analysis and/or Interpretation - S.S., O.A.; Literature Search - S.S., O.A.; Writing Manuscript - S.S., O.A.; Critical Review - S.S., H.O., O.A.

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Financial Disclosure:** The authors declared that this study has received financial support by Gaziosmanpaşa University Scientific Research Project.

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