Eurasian J Med 2016; 48: 90-4 The Eurasian Journal of Medicine **Original Article**

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

Omer Atis[1], Semsettin Sahin[2], Koksal Ceyhan[3], Huseyin Ozyurt[2], Ali Akbas[2], Ismail Benli[2]

[1]Department of Medical Biology, Atatürk University School of Medicine, Erzurum, Turkey
[2]Department of Biochemistry, Gaziosmanpaşa University School of Medicine, Tokat, Turkey
[3]Department of Cardiology, Gaziosmanpaşa University School of Medicine, Tokat, Turkey

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; ε2, ε3, ε4 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 ε2 (%7,25), ε3 (%83,50), ε4 (%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 ε3 ve ε4 allellerine göre APOE düzeyi anlamlı olarak yüksekti (p<0,05), ε3 ve ε4 allelleri arasında ise faklılık 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 10th National Congress of Medical Genetics in December 2012, Bursa, Turkey.

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

Correspondence to: Omer Atis E-mail: dromeratis@hotmail.com

©Copyright 2016 by the Atatürk University School of Medicine - Available online at www.eurasianjmed.com

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. ε2, ε3 and ε4, 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, ε4 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 ε4 carriers are 8.3% higher than in ε3 homozygous individuals. LDL cholesterol concentrations in ε2 carriers, on the other hand, are 14.2% lower than in ε3 homozygous individuals [13-15]. It has been shown by different studies that individuals carrying ε2 has 40% higher CAD death risk than individuals with *APOE3/3* genotype or individuals carrying ε2 [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 Cycler 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	
APOE (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^2)	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; APOE: 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 ε3 allele, ε2 allele was significantly associated with low LDL cholesterol levels. On the other hand, ε4 allele was associated with higher LDL cholesterol levels [22]. Data from the Turkish Heart Study revealed that ε2 alleles were associated with lower total and LDL cholesterol [8]. Considering the lipid levels and allele distributions, total and LDL cholesterol levels of ε2 allele in CAD and control group were lower than those of ε3 and ε4 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 ε3 and ε4 alleles. As the average of all study groups, ε4 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 ε3, ε4 and ε2 alleles were determined to be 86.0, 7.9 and 6.1%, respectively [9]. In this study, frequencies of ε3, ε4 and ε2 alleles in CAD patients were 85.5, 8.0 and 6.5%, respectively. Frequencies of ε3, ε4 and ε2 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 ε2 allele is associated with higher levels of APOE, whereas ε4 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 ε2 and lowest in ε4 alleles in all study groups, but the difference was more pronounced in CAD group. There was no difference between ε3 and ε4. 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 ε4 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.

References

1. Lenzen HJ, Assman G, Buchwalsky R, Schulte H. Association of apolipoprotein E polymorphism, low-density lipoprotein cholesterol, and coronary artery disease. Clin Chem 1986; 32: 778-81.
2. Lambert JC, Brousseau T, Defosse V, et al. Independent association of on APOE gene promoter polymorphism with increased risk of myocardial infarction and decreased APOE plasma concentrations -the ECTIM study. Hum Mol Genet 2000; 9: 57-61. [CrossRef]
3. Brazier L, Tiret L, Luc G, et al. Sequence polymorphisms in the apolipoprotein (a) gene and its association with lipoprotein (a) levels and myocardial infarction. The ECTIM Study. Atherosclerosis 1999; 144: 323-33. [CrossRef]
4. Kuivenhoven JA, Jukema JW, Zwinderman AH, et al. The role of a common variant of the cholesteryl ester transfer protein gene in the progression of coronary atherosclerosis. The Regression Growth Evaluation Statin Study Group. N Engl J Med 1998; 338: 86-93. [CrossRef]
5. Eichner JE, Dunn ST, Perveen G, Thompson DM, Stewart KE, Stroehla BC. Apolipoprotein E polymorphism and cardiovascular disease: a HuGE review. Am J Epidemiol 2002; 155: 487-95. [CrossRef]
6. Bhatnagar D, Durrington PN. Does measurement of apolipoproteins add to the clinical diagnosis and management of dyslipidaemias. Curr Opin Lipidol 1993; 4: 299-304. [CrossRef]

7. Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 1988; 240: 622-30. [CrossRef]
8. Mahley RW, Paloğlu KA, Atak Z, et al. Turkish Heart Study: lipids, lipoproteins, and apolipoproteins. J Lipid Res 1995; 36: 839-59.
9. Scott J, Knott TJ, Shaw DJ, Brook JD. Localization of genes encoding apolipoproteins CI, CII, and E to the p13----cen region of human chromosome 19. Hum Genet 1985; 71: 144-6. [CrossRef]
10. Hanis CL, Hewett-Emmett D, Douglas TC, Bertin TK, Schull WJ. Effects of the apolipoprotein E polymorphism on levels of lipids, lipoproteins, and apolipoproteins among Mexican-Americans in Starr County, Texas. Arterioscler Thromb 1991; 11: 362-70. [CrossRef]
11. Eichner JE, Kuller LH, Orchard TJ, et al. Relation of apolipoprotein E phenotype to myocardial infarction and mortality from coronary artery disease. Am J Cardiol 1993; 71: 160-5. [CrossRef]
12. de Andrade M, Thandi I, Brown S, Gotto A Jr, Patsch W, Boerwinkle E. Relationship of the apolipoprotein E polymorphism with carotid artery atherosclerosis. Am J Hum Genet 1995; 56: 1379-90.
13. Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 1988; 8: 1-21. [CrossRef]
14. Schaefer EJ, Lamon-Fava S, Johnson S, et al. Effects of gender and menopausal status on the association of apolipoprotein E phenotype with plasma lipoprotein levels. Results from the Framingham Offspring Study. Arterioscler Thromb 1994; 14: 1105-13. [CrossRef]
15. Hallman DM, Boerwinkle E, Saha N, et al. The apolipoprotein E polymorphism: a comparison of allele frequencies and effects in nine populations. Am J Hum Genet 1991; 49: 338-49.
16. Stengard JH, Weiss KM, Sing CF. An ecologicalstudy of association between coronary heart disease mortality rates in men and there lative frequencies of common allelic variations in the gene coding for apolipoprotein E. Hum Genet 1998; 103: 234-41. [CrossRef]
17. Minihane AM, Jofre-Monseny L, Olano-Martin E, Rimbach G. ApoE genotype, cardiovascular risk and responsiveness to dietary fat manipulation. Proc Nutr Soc 2007; 66: 183-97. [CrossRef]
18. Kulminsi AM, Arbeev KG, Culminskaya I, et al. Age, gender, and cancer but not neurodegenerative and cardiovascular diseases strongly modulate systemic effect of the Apolipoprotein E4 allele on lifespan. PLoS Genet 2014 30; 10: 1004141.
19. Attila G, Acartürk E, Eskandari G, et al. Effects of apolipoprotein E genotypes and other risk factors on the development of coronary artery disease in Southern Turkey. Clin Chim Acta 2001; 312: 191-6. [CrossRef]
20. Haas MJ, Mooradian AD. Regulation of high-density lipoprotein by inflammatory cytokines: establishing links between immune dysfunction and cardiovascular disease. Diabetes Metab Res Rev 2010; 26: 90-9. [CrossRef]
21. Okuyama H, Hamazaki T, Ogushi Y. Committee on Cholesterol Guidelines for Longevity, the Japan Society for Lipid Nutrition. New Cholesterol Guidelines for Longevity (2010). World Rev Nutr Diet 2011; 102: 124-36. [CrossRef]
22. Marrzoq LF, Sharif FA, Abed AA. Relationship between ApoE gene polymorphism and coronary heart disease in Gaza Strip. J Cardiovasc Dis Res 2011; 2: 29-35. [CrossRef]
23. Singh PP, Singh M, Mastana SS. APOE distribution in world populations with new data from India and the UK. Ann Hum Biol 2006; 33: 279-308. [CrossRef]
24. Siest G, Pillot T, Régis-Bailly A, et al. Apolipoprotein E: an important gene and protein to follow in laboratory medicine. Clin Chem 1995; 41: 1068-86.
25. Hanis CL, Hewett-Emmett D, Douglas TC, Bertin TK, Schull WJ. Effects of the apolipoprotein E polymorphism on levels of lipids, lipoproteins, and apolipoproteins among Mexican-Americans in Starr County, Texas. Arterioscler Thromb 1991; 11: 362-70. [CrossRef]
26. Cordan J, Yeşilbursa D, Baran İ, Güllülü S. Kardiyoloji. Uludağ Üniversitesi Basımevi Müdürlüğü 2005; 251-8.