Relationship between severity of coronary artery disease and apolipoprotein E gene polymorphism

Koroner arter hastalığı şiddetinin apolipoprotein E gen polimorfizmi ile ilişkisi

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ABSTRACT

Objective: To explore the possible contribution of the apolipoprotein (apo) E polymorphisms to the extent and severity of coronary artery disease (CAD) related to lipid metabolism.

Methods: Overall, 53 Turkish patients, aged 54±11years defined by coronary angiography were included in this cross-sectional study. Reardon's coronary artery scoring was used. Serum lipids were measured with enzymatic colorimetric methods. Apolipoproteins were measured with nephelometry. Apolipoprotein E gene polymorphisms were determined by the reverse hybridization method. Statistical analyses were performed using one-way ANOVA, Kruskal-Wallis and Chi- square tests.

Results: The genotype frequencies were 7.5% for E2/E3, 77.4% for E3/E3 and 15.1% for E3/E4. The E2 allele frequency was slightly lower than E4 allele. There were no significant differences between apo E2/E3, E3/E3 and E3/E4 genotypes for severity scorings (26, 41 and 32 respectively, p=0.30) and extent scorings (3.2, 5.5, 4.5, p=0.17). It was found that the most of patients who had E2/3 and E3/4 alleles had low severity scores. On the other hand, there were no significant score difference for patients who had E3/3 alleles. Lipids were not significantly different among the different genotypes. The E3 allele was associated with high apo B levels compared with E2 and E4 genotypes. It was found that severity and extent of disease were not related with lipid metabolism.

Conclusion: We concluded that there were no statistically significant differences between genotypes for extent and severity scorings, but the apo E3 allele is associated with more severe disease than E2 allele. These associations with severity were mediated not only by changes in lipid metabolism but may be also by other mechanisms in CAD patients. *(Anadolu Kardiyol Derg 2010; 10: 202-8)*

Key words: Coronary artery disease, polymorphism, apolipoprotein E, lipoprotein

ÖZET

Amaç: Apolipoprotein (apo) E polimorfizmlerinin lipid metabolizması ile de ilişkili olarak koroner arter hastalığının yaygınlık ve şiddetine olan olası katkısını tespit etmek.

Yöntemler: Koroner anjiyografi ile tanıları konmuş, yaşları 54±11 olan 53 Türk hasta bu enine-kesitsel çalışmaya alındı. Reardon'ın koroner arter skorlaması kullanıldı. Serumda lipidler enzimatik kolorimetrik yöntemle ölçüldü. Apolipoproteinler nefelometri ile ölçüldü. Apolipoprotein E gen polimorfizmi *reverse-hybridization* yöntemi ile tespit edildi. İstatistiksel analizde tek-yönlü ANOVA, Kruskal-Wallis ve Ki-kare testleri kullanıldı.

Bulgular: Genotip sıklıkları E2/E3 için %7.5, E3/E3 için %77.4 ve E3/E4 için %15.1 olarak bulundu. E2 allel sıklığı E4 alleline göre daha düşüktü. İstatistiksel olarak apo E2/E3, E3/E3 ve E3/E4 genotipleri arasında şiddet skorları (26, 41 ve 32 sırasıyla, p=0.30) ve yaygınlık skorları (3.2, 5.5, 4.5, p=0.17) açısından anlamlı fark bulunmadı. Apolipoprotein E2/3 ve E3/4 allellerine sahip hastaların çoğunda düşük şiddet skorları bulunurken, diğer yanda E3/3 alleline sahip hastalarda anlamlı skor farkı yoktu. Lipidler değişik genotipler arasında farklılık göstermedi. E2 ve E4 genotiplerine nazaran E3 alleli yüksek apo B seviyeleri ile birlikteydi. Hastalık şiddet ve yaygınlığı lipid metabolizması ile ilişkili bulunmadı.

Sonuç: Hastalığın yaygınlık ve şiddetinde genotipler arasında istatistiksel olarak anlamlı fark görülmediği sonucuna varıldı, fakat apo E3 alleli E2'ye göre hastalığın daha şiddetli oluşu ile birlikteydi. Koroner arter hastalığında hastalığın şiddeti sadece lipid metabolizması ile ilişkili olarak değil diğer mekanizmalar ile de açıklanabilir. *(Anadolu Kardiyol Derg 2010; 10: 202-8)*

Anahtar kelimeler: Koroner arter hastalığı, polimorfizm, apolipoprotein E, lipoprotein

Introduction

Coronary artery disease (CAD) is the leading cause of death and premature disability. CAD is a complex disorder resulting from many risk factors. Individuals with genetic predisposition to atherosclerosis have substantial risk for developing CAD, especially at early ages (1). Turkish adults have low levels of total cholesterol (mean 185 mg/dl), low-density lipoprotein (LDL) cholesterol (mean 116 mg/dl), high-density lipoprotein (HDL) cholesterol (mean 37 and 45 mg/dl in men and women), triglycerides (mean 143 mg/dl), apolipoprotein (apo) B (mean 115 mg/dl) (2) and have a high prevalence of CAD (3).

While it is difficult to explore the relation between local vessel wall function and CAD severity, measuring DNA variants such as apo E polymorphisms may provide a way to assess this link because of its known effect on endothelial cell proliferation (4).

Apolipoprotein E is a plasma glycoprotein and a member of the apo gene family. It is located at chromosome 19q13.2, and consists of four exons and three introns spanning 3.597 nucleotides, and produces a 299 amino acid polypeptide with a molecular mass of about 34 kDa (5). These isoforms differ in amino acid sequence at positions 112 and 158. Apo E3 contains cysteine at 112 and arginine at 158. Apo E2 has cysteine at both positions, and E4 has arginine at both sites. While there are rare variants, among the variants of this gene, alleles E2, E3, and E4 constitute the common polymorphism found in most populations in relation to cardiovascular disease. Apo E3 is the most frequent (>60%) in all populations studied. From these alleles arise six phenotypes; their ranking from most to least common is generally 3/3, 4/3, 3/2, 4/4, 4/2, and 2/2 (5, 6). The apo E locus harbors one of the genes that are involved in the control of plasma lipid levels, accounting for about 10% of the total variation in cholesterol levels (7).

Many studies have shown that apo E polymorphism may enhance atherogenesis indirectly by a strong effect on circulating levels of LDL cholesterol and apo B (8-11). Recent reports suggest that the apo E gene could have a direct effect on the response of the arterial wall to injury (12, 13). Each of biochemical process associated with CAD comprises enzymes, receptors and a ligand, which are encoded by genes. Variations in these genes can alter the function of the constituents within a metabolic pathway. These genetic variations interact with each other and with nongenetic factors, resulting in variable susceptibility to the development and progression of atherosclerosis and thrombosis.

The determination of blood lipid and lipoprotein levels in CAD is a commonly investigated subject because of it is one of the coronary risk factors. In terms of cardiovascular diseases, polymorphisms at multiple genes have been associated with differential effects in terms of lipid metabolism; however, the connection with cardiovascular disease has been more elusive, and considerable heterogeneity exists among studies (14). However, age, gender, uric acid and smoking were not effective on diseased vessel extent, both LDL cholesterol and HDL cholesterol scores decreased with increased number of involved vessels (15). Thus, apo E polymorphism could be associated with CAD severity and extent by mechanisms related to both circulating lipids, lipoproteins and apolipoproteins.

The aim of our study is to investigate the role of polymorphisms of apo E gene in CAD defined by coronary angiography and assess the findings in relation to the severity and extent of disease in Turkish patients.

Methods

Patients

We studied 53 patients aged 54±11 years, both men (n=36, mean age; 54±10 years) and women (n=17, mean age; 55±14 years), consecutively referred to coronary intensive care unit of the Department of Cardiology due to an acute myocardial infarction (MI) and who underwent coronary angiography during follow up periods (from January 2006 to July 2006) in this cross-sectional study. Therefore, the patients who have unstable angina pectoris were not included to the study because of their primary treatment approach was medical treatment and angiography decision would be undergone later.

Table 1 provides clinical and demographic characteristics of patients. Written informed consent was obtained from each participant before inclusion in the study. The procedures used were in accordance with the guidelines of the Helsinki Declaration on human experimentation. Body mass indexes of patients were calculated as 27.5±4.5 kg/m^2 in men and 26.5±4.4 kg/m^2 in women.

DNA analysis for the detection of apo E genotypes

A 2-mL venous blood sample was drawn into an EDTA sample tube before the angiogram in a period of 24 hours. The genotypic structure was detected by CVD StripAssay method that works with a reverse hybridization principle followed by performing PCR. Catalog number of the commercial kit was 4-240/4-241 (ViennaLab, Vienna, Austria).

In the first step of the study, total genomic DNA was extracted from peripheral anticoagulated blood mononuclear cells by a rapid and convenient procedure. In the second step, relevant gene sequences are simultaneously in vitro amplified and biotin-labeled in a single (®multiplex) amplification reaction. Finally, the amplification products are selectively hybridized to a test strip, which contains allele-specific (wild type and mutant) oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and color substrates.

Lipoprotein analysis

Total cholesterol (TC), HDL cholesterol and triglyceride levels were measured by enzymatic colorimetric methods (Architect C800, Abbott Diagnostics, USA). The LDL cholesterol levels were calculated using the Friedewald formula. We measured levels of apo AI and apo B using nephelometric method (Delta nephelometry, Seac Diagnostics, Italy).

Determination of CAD extent and severity

Following diagnosis of MI, anticoagulant and/or thrombolytic therapy was commenced. The angiograms were assessed by

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cardiologists who were unaware that the patients were to be included in the study.

According to the results of the coronary angiography, the coronary artery scoring was performed in order to evaluate the extent and severity of atherosclerosis providing a numerical value for lesions. This scoring method (16) was proposed by Reardon et al. with a modification of the coronary atherosclerosis scoring system described previously (17).

In that study, the coefficient of variation between two angiograms was analyzed several months apart without knowledge of the previous score was 4.9%. According to Reardon scoring system, for analysis the coronary circulation was divided into eight proximal segments. Disease in the distal segments was not considered because of difficulty in quantitation of the lesion severity in these areas.

Each angiogram was classified as revealing either lesion was on the left coronary artery (anterior inter-ventricular artery-upper, middle and bottom segments and circumflex artery-upper and bottom segments) or right coronary artery (upper and bottom segments).

The severity of CAD was determined as follows. Total severity number was calculated from additional scores of lesions. Severity numbers of normal vessel, coronary lesion with >50%, 50-75%, 76-89%, 90-99%, 100% luminal stenosis were 0, 10, 15, 20, 25 points, respectively. Total extent scoring was calculated from additional scores of lesions. Extent scores of normal vessel, lesion isolated in side branch of main vessel, lesion isolated in main vessel, diffuse lesion in main vessel, diffuse lesion in common artery with side branch were 0, 0.5, 1, 2, and 2.5 points, respectively. These provide a numerical value for lesion extent and severity.

Statistical analysis

All analyses were performed using Statistical Package for Social Sciences statistical package (SPSS, version 11.0 for Windows XP, Chicago, IL,USA). To compare independent groups, one-way ANOVA and Kruskal Wallis tests were performed to compare continuous variables and Pearson Chi- square analysis was performed to compare categorical variables. Spearman correlation analysis was performed to assess the relationships among numeric variables.

Statistical power, standard deviation of means (Sm) and standard deviation (S) of this study were 0.184, 4.05 and 22.65 for severity scores; 0.265, 0.42, and 1.91 respectively for extent scores; performed by one- way ANOVA power analysis.

The parametric one-way ANOVA analysis, observing variance homogeneity and normal distribution for age and body mass index values (Table 1) for lipid levels (Table 4) and Pearson Chi-square test for other qualitative data were performed (Table 1). Because mean and median values were distant from each other, the non-parametric Kruskal-Wallis test (Table 2) and Spearman correlation test (Table 5) were performed for extent and severity scores. The odds ratios were calculated for scorings, thus Pearson Chi- square test was performed (Table 3). P values <0.05 were considered statistically significant. To assess the association between genotype and severity, odds ratios (ORs) with 95% confidence intervals (CIs) were calculated.

Results

The apo E2, E3 and E4 allele frequencies in patients were found to be as 6%, 82% and 12% and E2/E3, E3/E3 and E3/E4 genotype frequencies were as follows: 7.5%, 77.4 % and 15.1%, respectively.

Table 2 shows genotype frequency of apo E gene polymorphisms according to the severity and extent scores of lesions. Table 3 shows genotype frequency of apo E gene polymorphisms according to the extent scores (scores ≤5 or >5) of lesions.

Serum lipid parameters of patients according to their genotypes of apo E gene polymorphism are shown in Table 4. Relationships of lipid parameters with diffusion and severity of coronary artery disease are demonstrated in Table 5.

Patients who had E3 allele had higher mean levels of apo B (p=0.03) and a trend of higher mean levels of TC, triglyceride, and LDL cholesterol, but these differences were not significant. There were also no significant relations between the apo E gene polymorphisms and CAD extent and severity in our study. We also found no relations between apo E genotypes and diabetes, hypertension, positive family history of premature CAD, or the presence and severity of angina. It is possible that these parameters were affected by antihyperlipidemic, anticoagulant and thrombolytic therapies or difficulties in standardization of the nutritional properties.

Discussion

In this study, we investigated the role of polymorphisms of apo E gene in Turkish patients with CAD and probable differences between genotypes for extent and severity scorings. It was shown that there were also no significant relations between the apo E gene polymorphisms and CAD extent and severity; however, the apo E3 allele is associated with more severe disease than E2 allele. Similarly, it was shown that apo E polymorphism was not associated with the number of coronary vessels with significant obstruction at any age range. On the other hand, dyslipidemia associated with the multivascular lesion (18).

Apolipoprotein E gene polymorphisms and CAD

Apo E gene polymorphisms are associated with atherosclerosis and play critical roles in lipid metabolism. The lipid levels were observed to increase following apo E genotype with the order E3/2, E3/3, E3/4, E4/4 in male patients with coronary artery disease (19-21).

Some researchers found low levels of apoB in apo E2 homozygotes and high levels in patients who possess apo E4 allele. According to those studies, apo E polymorphism may affect LDL cholesterol and apo B levels and cause an increase in atherogenesis formation (22, 23).

It was observed that individuals who possess apo E4 had more severe types of coronary artery disease and also experienced

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Table 1. Clinical and demographic characteristics of patients

Variables	All patients	E2/E3	E3/E3	E3/E4	p
n	53	4	41	8	
Age, years	54±11	46±7	55±11	51±10	0.216*
Sex, female	17	2	14	1	0.354**
BMI, kg/m^2	27±4	28±4	27±4	27±3	0.797*
HT, N	22	0	20	2	0.099**
Smoking, n	34	3	26	5	0.894**
Alcohol, n	3	0	3	0	0.628**
DM, n	16	1	12	3	0.874**
Hyperlipidemia, n	12	1	11	0	0.251**
CNS disease, n	1	0	1	0	0.861**
CVD disease, n	17	1	12	4	0.492**
Familial CNS, n	8	0	7	1	0.645**
Familial CVD, n	26	0	23	3	0.078**
Familial DM, n	9	0	8	1	0.572**
Familial HT, n	16	0	13	3	0.372**
Familial HL, n	8	0	7	1	0.522**
Medications					
antihypertensives, n	11	0	10	1	0.255**
hypolipidemics, n	7	1	6	0	0.365**
aspirin, n	10	1	7	2	0.211**

Results are shown as mean ± SD and proportions
*One-way ANOVA and **Pearson Chi-square tests
BMI - body mass index, CNS - central nervous system, DM - diabetes mellitus, HL - hyperlipidemia, HT - hypertension

Table 2. Apolipoprotein E genotype frequency according to severity and extent scores

Apolipoprotein E genotypes	n	%	Extent	Severity
E2/E3	4	7.5	3.25 (2-6)	26 (21-51)
E3/E3	41	77.4	5.5 (1-10.5)	41(4-115)
E3/E4	8	15.1	4.5 (2-6.5)	32.5 (4-80)
p			0.17*	0.30*

Data are presented as numbers/percentages and median (min-max) values
*Kruskal-Wallis test

Table 3. Apolipoprotein E genotype frequency according to severity scores

Apolipoprotein E genotypes	Extent≤5 score		Extent>5 score		p*	OR 95% CI
	n	%	n	%		
E2/3	3	75.0	1	25.0	0.198	4.23** (0.40-44.2)
E3/3	17	41.5	24	58.5		
E3/4	5	62.5	3	37.5	0.274	0.42** (0.08-2.02)

Data are presented as numbers/percentages and OR (95%CI) values
*Pearson Chi-Square test, ** - compared with E3/3 genotype of apo E
 CI - confidence interval, OR - odds ratio

infarcts more frequently than others in worldwide (5, 21, 23). In agreement with those reports, according to the meta-analysis study, carriers of the apo E4 allele had a 42% higher risk for CAD compared to E3 allele carriers (24).

Allele frequencies

It has been seen that allele as well as genotype frequencies differ among populations. In Uygur population, the frequencies of the E2, E3, and E4 were found to be 15%, 64%, and 19% and in Han population, those ratios were 8%, 77%, 14% respectively (25). The frequencies of alleles E3 and E4 were similar in Russia and neighboring countries (26).

The E2, E3 and E4 allele incidences of 240 Inner and Western Anatolia originated Turkish people living in Germany were found as 4.8%, 88% and 6.6%, respectively (27). Apo E genotypes in a total of 8366 participants from seven different localities of Turkey were identified and apo E3 was found to be the most common genotype. Apo E4 and E2 allele incidences were 7.9% and 6.1%, respectively (28).

This study was performed in Western region of Turkey. Allele frequencies were detected by additive alleles of each genotypes and calculating percentages. The frequencies of the apo E2, E3 and E4 alleles were 6%, 82% and 12%, respectively, similarly to the study performed in Southern region (29). The order of the frequency of apo E alleles (E3→ E4→E2) in Turkish population was similar to most populations in the world.

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Table 4. Serum lipid and apolipoprotein levels of patients according to their apo E genotypes

Variables	All patients	E2/E3	E3/E3	E3/E4	p*
n	53	4	41	8	
Frequency, %		7.5	77.3	15.0	
Total cholesterol, mg/dl	199±44	190±42	204±43	179±49	0.32
Triglyceride, mg/dl	201±125	162±53	216±136	143±60	0.26
HDL cholesterol, mg/dl	41±7	40±3	42±7	38±5	0.32
LDL cholesterol, mg/dl	117±37	117 ±31	118±37	112±43	0.92
n	40	3	31	6	
Frequency, %		7.7	76.9	15.4	
Apo AI, g/L	1.09±0.23	1.11±0.18	1.09±0.24	1.07±0.25	0.96
Apo B, g/L	1.0±0.31	0.86±0.1	1.04±0.33	0.87±0.32	0.03

Data are presented as mean±SD
*One-way ANOVA test
Apo - apolipoprotein, HDL - high-density lipoprotein, LDL - low-density lipoprotein

Table 5. Relationships of lipid parameters with extent and severity of coronary artery disease

n=53	Extent	Severity
	p (r)*	p (r)*
Total cholesterol	0.30 (0.142)	0.09 (0.235)
Triglyceride	0.35 (0.129)	0.26 (0.158)
HDL cholesterol	0.67 (-0.059)	0.23 (0.167)
LDL cholesterol	0.36 (0.128)	0.52 (0.090)
n=40		
Apo A1	0.84 (-0.033)	0.75 (0.050)
Apo B	0.79 (0.042)	0.19 (0.211)

*Spearman's rank correlation test
Apo - apolipoprotein, HDL - high-density lipoprotein, LDL - low-density lipoprotein

Associations with lipid levels

In the present study, it was demonstrated that no significant differences existed among different genotypes of apo E gene polymorphism in terms of serum TC, triglyceride, HDL cholesterol, LDL cholesterol and apo AI levels (except apo B levels) and the extent and severity of the CAD. Similarly, in Greek patients with familial hypercholesterolemia (30) and Mexican patients with CAD (31), apo E polymorphism was not associated with lipid levels and CAD. However, the most common apo E polymorphism has been found to influence blood lipid concentrations and its correlation with CAD has been extensively investigated in the last decade. In Western Iran, a significant association between apo E polymorphism and the level of plasma lipids in patients with CAD was demonstrated (32).

In results of this study did not confirm some previous reports' findings; this may be because of experimental limitations such as study's small size, the phenotype complexity, and the interactions with environmental factors. Those complexities necessitate future research in the field on dietary advice optimized for the individual's genome.

Total cholesterol, LDL cholesterol and apo B levels were found low in patients who possess apo E2 allele whereas high in patients who possess apo E4 allele, although there were no correlations between CAD and alleles (29). On the contrary, some researchers did not find any relationships between apo E alleles and lipid levels (33). On the other hand, there are some other studies where the apo E4 allele was demonstrated to be a risk factor for atherosclerosis, increased total cholesterol and LDL cholesterol levels were suggested to determine the severity of CAD in patients who have apo E4/E4 genotype (34). In a meta-analysis study, linear relationships of apo E genotypes with coronary risk were reported (35). Among Omani dyslipidemic patients who had CAD, carriers of apo E4 compared to E3 had significantly higher levels of LDL cholesterol and apo B without relationship with CAD (36). The bearers of E3/E4 genotype had three-fold higher propensity of developing CAD in the population of Northwest India (37).

It was also shown that in female patients who underwent coronary artery bypass surgery, levels of apo B and apo AI were related with extent of stenosis and the number of significantly diseased vessels (38).

Study limitations

There were some limitations of the study such as low sample size due to the cost problems.

Although sample size was not enough for high statistical power; total sample size of the group was supposed to be 266 for severity scores; 173 for extent scores for 0.80 statistical power, this study may provide directions for further research on the subject.

Conclusion

In the present study, it was found that extent and severity of disease were not related with circulating lipids, lipoproteins, apolipoproteins, total cholesterol/HDL cholesterol and LDL cholesterol/HDL cholesterol ratios. We conclude that among patients with CAD, the apo E3 allele is associated with more

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severe and the E2 allele with less severe disease. We also showed protective effects of E2 on CAD. In addition, our data suggest that the role of this polymorphism in determining the lipid profile cannot be excluded. These associations with severity were mediated not only by changes in circulating lipids, lipoproteins and apolipoproteins but also by other mechanisms in this population of CAD patients. The variability and conflicting data of those studies may be due to geographic and ethnic background, differences of allele frequencies, sex, laboratory characteristics and study designs. Further studies with high number of population and seeking for different mutations would help to understand the genetic background of CAD.

Conflict of interest: None declared

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