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Original Article

Relation of apolipoprotein E gene polymorphism with the severity of coronary artery disease in patients with stable ischemic heart disease

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Abstract

Aim: Atherosclerosis begins from an early age and manifests in later years as Coronary artery disease (CAD). This inflammatory process is aggravated by age, smoking, hypercholesterolemia, hypertension, diabetes mellitus, and genetic factors. We aimed to investigate which isoform of APOE is related to extensive coronary lesions in patients with stable coronary heart disease.

Materials and Methods: This study was carried on single center. One hundred and ten patients diagnosed with stable coronary artery disease by coronary angiogram were enrolled consecutively. Syntax score was calculated by a tool of website calculator (www.syntax.com). According to the Syntax score, patients were split into three groups. APOE genotyping was performed through blood samples. Patients split into three groups according to the APOE genotypes: E4 (3/4 and 4/4 genotypes), E3(3/3 genotype), E2 (2/2 and 2/3 genotypes). APOE groups were compared according to baseline characteristics and syntax scores.
(%82.6) değil (82.6%) olacak. Lütfen İngilizce kurala göre düzeltiniz. Tüm sayısal değerlerde virgülleri de nokta yapmayı unutmayınız.

Results: Coronary angiography and APOE genotypes of 98 patients were analyzed. 81 of patients (%82.6) had E3E3 allele; 6 of patients (%6.1) had E2E3 allele; 10 patients (%10.2) had E3E4 allele and 1 patient (%1) had E2E4 allele. Due to the contrast effect of E2 and E4 on CAD, we excluded patients with E2E4 allele from the study. Firstly, we assessed distribution of APOE genotype E2 (E2E3), E3 (E3E3 and E3E4), E4 (E3E4) within 3 groups of syntax scores. Total of 6 patients of E2 allele was at low syntax score group. 83 patients of E3 allele were at the low-risk group of syntax score. 10 patients of E3 allele were at the mid group and 4 patients were at the high-risk group of syntax score. 7 patients of E4 allele subjects were at the low-risk and 1 patient was at the high-risk group of syntax score. Compared to syntax score groups and APOE genotypes, E2 alleles were in lower syntax score group versus E3 (P=0.046) and E4 (P=0.003) alleles. However E4 alleles were in higher syntax score group versus E3 alleles (P= 0.034). The Syntax score was seemed to be lower in the E2 allele group versus E4 and E2 groups (P=0.013).

Conclusion: we reported the first study that E2 allele was related with less and E4 allele was more extensity and severity of CAD in patients with stable ischemic coronary disease

Keywords: APO E; Stable Ischemic Coronary Disease; SYNTAX Score

INTRODUCTION

Coronary artery disease (CAD) is known to be the most common cause of death worldwide. CAD risks are multifactorial and consist of environmental and genetic factors (1). Atherosclerosis begins from an early age and manifests in later years as CAD. This inflammatory process is aggravated by age, smoking, hypercholesterolemia, hypertension, diabetes mellitus, and genetic factors. It is possible to reduce the death rate from 40% to 30% through taking precautions against known risk factors (2,3). Recent studies revealed that genetic susceptibility may contribute around 50% risk burden on CAD. 10 of known 36 genes contributing to CAD are relevant to hypertension and blood lipid levels. Death rate may decrease more than 40% by managing environmental and genetic factors (4–8). Lipids and lipoproteins play a major role in developing CAD and Apolipoprotein E (APOE) is the ligand of its receptors of lipoprotein. The low-density lipoprotein (LDL) receptors and Apolipoprotein E specific receptors found

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on the liver and other tissues are essential for cholesterol uptake. APOE is the main part of these two receptors. It regulates lipoprotein bindings to LDL and lipoprotein remnant receptors and play a major role of distribution of cholesterol in various cells of body and liver. A defect of this protein leads unbinding total and LDL cholesterol to protein and elevated blood cholesterol level (9,10). Recent studies revealed that even without the increasing levels of plasma cholesterol in itself, the APOE gene was found related with atherosclerosis and coronary artery disease eventually (11–14).

APOE gene includes 299 amino acid and located on chromosome. This gene codes three alleles of APOE : E2, E3, E4 and 6 phenotypes: APOE 2/2; 2/3; 2/4; 3/3; 3/4; 4/4. The most common type of allele is E3 (>75%), E4(15%) and E2(8%) respectively (10,15,16). E4 alleles was found to be related with higher blood concentration of LDL, atherosclerotic burden and CAD, ultimately (17,18). However epidemiological studies have shown poor relation with clinically defined atherosclerosis, APOE variation has an impact on CAD outcome (19).

Syntax score (Synergy between Percutaneous Coronary Intervention with TAXUS and Cardiac Surgery) has been developed as a comprehensive angiographic scoring tool for quantification of coronary lesions with respect to their number, location, and complexity. At first, it was used to allocate PCI or CABG (coronary artery by pass surgery) in patients with stable coronary artery disease, multivessel or complex coronary lesions. Afterward this score was started to use in all involved patients underwent PCI (Percutan Coronary Intervention) (20).

In this study we aimed to demonstrate which isoform of APOE has more extensive coronary lesions in patients with stable coronary heart disease(SHD).

MATERIALS and METHODS

Study design

110 patients with SHD determined by coronary angiogram are consecutively included. Patients with acute coronary syndrome, heart failure, moderate to severe heart valve disease, previous coronary artery disease, chronic kidney disease, liver disease, coronary stenosis of less than 50%, and familial hypercholesterolemia are excluded from the study. Prior to procedure, blood sample obtained from all patients. The Syntax score was calculated by a tool of website calculator (www.syntax.com) from baseline angiograms, for all coronary lesions with a diameter stenosis greater than 50% in vessels larger than 1.5 milimeter (mm). All angiographic variables were evaluated by two cardiologists. According to the syntax score patients split into three groups. (Table 1A) APOE genotyping was performed through blood samples and patients split into three groups: E4 (3/4 and 4/4 genotypes), E3(3/3 genotype), E2 (2/2 and 2/3 genotypes). (Table 1B) APO E groups and syntax groups were compared accordings to distribution. (Table 2). Due to the adverse effect of E2 and E4 alleles to eachother on lipid metabolism, we lifted subjects who have E2E4 allele. Finally, the study consisted of 97 individuals.

Table 1A. Clinical and laboratory characteristics of patients in groups of apoe alleles

	APO E2 (n)	APO E3 (n)	APO E4 (n)	P value
Age, years	70.3±11.2	63.65±9.9	60.9±8.6	0.183
Male gender	5	67	5	0.053
BMI, kg/m²	25.1±4.4	28.73±4.5	28.2±2.5	0.159
Hypertension	5	51	6	0.583
Diabetes Mellitus	0	30	5	0.119
Hypercholestrolemia	3	46	6	0.881
Total cholestrol, mg/dL	186±42.22	202.69±58.75	194.40±36.11	0.728
Triglycerides, mg/dL	152.33±72.35	181.51±109	185.7± 82.04	0.794
LDL cholestrol, mg/dL	105.5±27.5	133.07±39.55	119.3±25.76	0.152
HDL cholestrol, mg/Dl	43.8±13.6	42.68±8.5	40.9±9.8	0.790

SS: Syntax Score; BMI: Body Mass Index; LDL: low-density lipoprotein; HDL: high-density lipoprotein

Table 1B. Clinical and laboratory characteristics of patients in syntax score groups				
	SS 0-23 (n)	SS 23-32 (n)	SS > 32 (n)	p value
Age, years	63.05±9.9	68.8±10.5	66.5±7.8	0.198
Male gender	67	9	1	0.018
BMI, kg/m²	28.68±4.5	26.25±3	29.18±3.59	0.241
Hypertension	57	2	3	0.009
Diabetes Mellitus	32	2	1	0.460
Hypercholestrolemia	44	4	3	0.485
Total cholestrol, mg/dL	199.04±52.5	205.7±83.2	225.25±48.54	0.633
Triglycerides, mg/dL	177.69±96.26	204.5±168.47	170±83.23	0.734
LDL cholestrol, mg/dL	128.19±36.2	138.7±57.75	144.5±18.04	0.533
HDL cholestrol, mg/dL	42.82±9.04	40.1±8.55	43.5±8.88	0.651
SS: Syntax Score; BMI: Body Mass Index; LDL: low-density lipoprotein; HDL: high-density lipoprotein				

Table 2. Apoe distribution in syntax score groups			
	SS<23	SS23-32	SS>32
E2, n(%)	6 (100%)	0	0
E3, n(%)	83 (85.5%)	10 (10.3%)	4 (4%)
E4, n(%)	7 (70%)	2 (20%)	1 (10%)
E2E3 , n(%)	6 (100%)	0	0
E3E3, n(%)	70 (96%)	8 (9.8%)	3 (37%)
E3E4, n(%)	7 (70%)	2 (20%)	1 (10%)
(E2 versus E3) : P = 0,046 (E3 versus E4): P= 0,034 (E2 versus E4): P= 0,003 SS: syntax score			

Clinical and laboratory data assessment

For each subject age, gender, length and weight are recorded. Body mass index (BMI) was calculated by tool of BMI per participant. The BMI was calculated with following formula: 'ratio of weight and height multiplied by 2' (21). Vascular risk factors including smoking, hypercholesterolemia, diabetes mellitus, hypertension, and familial history of coronary artery disease were enrolled. Diabetes mellitus was described as fasting blood glucose is upper than 126 mg/dL or individuals on antidiabetic therapy or blood glucose level is upper than 200 mg/dL at any time. Hypertension was defined as blood pressure is upper than 140/90 mm Hg 2 times on examination or on antihypertensive medication. Hypercholesterolemia was defined as the low-density lipoprotein (LDL) level is upper than 159 mg/dL or total cholesterol is upper than 199 mg/dL or individuals on antihyperlipidemic therapy (22). Individuals still smoke and former smokers who have quit smoking less than 3 months were considered in the category of current smokers Congestive heart failure (CHF) is accepted as ejection fraction (EF) is under 40%.

APOE Genotyping

Blood samples were obtained during the angiogram from patients in EDTA tube and reserved at 68 Fahrenheit (^{0}F) degree. Genomic DNA was excerpted from full blood cells by a QIAamp DNA purification kit (Qiagen, Germany). TaqMan method which is the method of Real-time polymerase chain reaction (PCR) was used for genotyping. TaqMan method is based on dual labeled oligonucleotide and exonuclease activity of Taq polymerase enzyme and have more specifity than SYBR Green method which is other real-time PCR method (23). Genotypes were allocated according to the combinations of the variant allelic forms as E2, E3, and E4.

Statistical Analysis

The SPSS 21.0 statistical software program (SSPS Inc, Chicago, Illinois) was used for the statistical calculation. Continuous variables were checked for normality by using Kolmogorov Smirnov test and data mentioned as the mean±standart deviation. Categorical datas presented as numbers and percentage and compared by chi-square test.

Student-t test and Mann-Whitney U test were performed to compare laboratory and clinical characteristics of patients according the datas appropriate. P <0.05 was accepted significant.

RESULTS

Total of 97 patients with their coronary angiogram and genotype of APOE analyzed. The demographic, baseline characteristics and syntax scores of patients are shown in Table 3, IIA, and IIB. The significant relations were not found including hyperlipidemia, diabetes mellitus, smoking status between syntax score groups and APOE genotype subgroups. Rate of hypertension was higher in the lower syntax group (p=0.009).

Table 3. Disribution of patients according to the apoe alleles

Genotype /allele	Count (%)
E2E3	6 (6.1)
E3E3	81 (82.6)
E3E4	10 (10.2)
E2E4	1 (1)
E3	81 (81.8)
E4	11 (11.1)
E2	7 (7.1)

Distribution of patients according to the APOE genotypes

81 of patients (%82.6) had E3E3 allele; 6 of patients (%6.1) had E2E3 allele; 10 patients (%10.2) had E3E4 allele and 1 patient (%1) had E2E4 allele. Due to the contrast effect of E2 and E4 on CHD, we excluded individuals with E2E4 allele from the study.

APOE genotype and lipid profile

We found no significance between APOE subgroups with serum LDL, TG, HDL and total cholesterol levels (p=0.287; p=0.734; p=0.651; p=0.665 respectively)

Syntax score and lipid profile (lipid profile and CAD)

There was no significance between syntax score with serum LDL, TG, HDL and total cholesterol levels. (p=0.533; p=0.084; p=0.844; p=0.633 respectively)

Relation of APOE genotype with syntax score

We divided patients into 3 groups for syntax score as low, mid and high. Distribution of APOE genotype E2 (E2E3), E3(E3E3 and E3E4), E4(E3E4) in 3 groups of syntax scores were compared. 6 subjects of E2 allele (100%) were in low syntax score group. 83 subjects of E3 allele (85.5%) were in low group of syntax score. 10 subjects of E3 allele (10.3%) were in the mid group and 4 subjects were in high group 4 (4%) of syntax score. 7 subjects of E4 allele were in low group (70%) and 1 of was in high group 1 (10%) of syntax score. The Following results were found when the syntax score was compared to the APOE genotypes: E2 alleles were in low syntax score group versus E3 (P = 0.046), and E4 (P=0.003) alleles ; and E4 alleles were in high syntax score group versus E3 alleles (P= 0.034). The Syntax score was seemed to be lower in E2 allele group versus E4 (P=0,013) (Table 2).

Gender and BMI effect on syntax score

There was no significant relationship between BMI with syntax score (P =0.391). BMI was not related with APOE genotype subgroups either (P =0.159). Rate of male gender was high in low syntax score group. (p=0.018)

DISCUSSION

We found that CAD severity and extensity of patients having E2 allele happened to be lower relative than of E3 and E4 aleles. Many studies have been carried out to determine the factors leading to CAD. Especially, in patients with SHD environmental risk factors were thought to play role in development of atherosclerosis less than acute coronary syndromes which put forward the genetic basis in patients with SHD and the APOE gene is one of the majors (9,10).

Atherosclerotic process starts an early age and is aggravated by environmental risk factors such as gender, smoking, hypercholesterolemia, hypertension, diabetes mellitus, and genetics. However, a large number of patients with SHD do not have traditional risk factors for cardiovascular disease. In these patients, the etiology may involve a genetic abnormality consistency. One of theese genetic factors is APOE. Apo E is one of the most major apolipoproteins in the central nervous system and showed significant associations with the cerebrovascular disease. APOE gene as a receptor-binding ligand mediating the clearance of chylomicron and remnants of very-low-density lipoprotein cholesterol from plasma also plays a major role in the metabolisms of cholesterol. APOE gene codes three alleles (E2, E3, E4) and 6 phenotypes (E2/E2; E2/E3; E2/E4; E3/E3; E3/E4; E4/E4) (10,17). Different epidemiologic studies have shown that APOE distribution is inconsistent in different populations. E3 allele and E3E3 genotype are the most common variant worlwide (24). In recent study, in seven different localities of Turkey, APOE3 allele is found the most common genotype. APOE4 allele incidence was found 7.9% and APOE2 allele incidence was 6.1% respectively (25). Other study found that Turkish people populating in Germany, APOE alleles distribution were following: E2: 4.8%; E3: 88%, and E4: 6.6% respectively (26). Our findings were very close to those of Turkish population living in Europe. The frequency of APOE4 allele in our patients were 11%, E3 allele rate was 81%, and E2 allele rate was 7.1% respectively (27). We carried out this study with Northern Anatolia of Turkey individuals and APOE distribution was more similar to Europeans than Inner and Western Anatolian originated people.

The Syntax Score (synergy between percutaneous coronary intervention [PCI] with TAXUS and cardiac

surgery) is a tool to score complexity and severity of coronary artery disease. It is calculated with the web tool and was found to related with long and short term outcome and MACE (Major Adverse Cardiovascular Events) in patients underwent PCI (20). We assessed the coronary artery disease extensity with syntax score in patients with stable heart disease (SHD). Given the previous reports E4 allele was found to have significant relation with CAD (17,18,28,29). In WISE study E4 was associated with both the number of diseased vessels and stenosis severity (30). In other study, acute myocardial infarction with E4 carriers and E3E4 genotype had higher severity scores compared to other APOE gene isoforms (31). This relation has not been studied in SHD patients yet.

However, E2 allele has defective receptor-binding ability and individuals have E2 allele expected to had lower circulating cholesterol levels and higher triglyceride levels and whereas carriers of the E4 allele appear to had higher plasma levels of total and low-density lipoprotein cholesterol compared to E3 carriers, in our study plasma cholestrol levels were similar between groups. We attribute this result to the small number of patients. Even so this is suggesting that APOE gene play role on development of CAD through not only cholesterol uptake but allele-specific antioxidant and immune activities. Apo E may play additional roles in the development of CHD through macrophage cholesterol efflux, platelet aggregation, and allele-specific antioxidant and immune activities. Further study is needed to understand the effects of these alleles on CAD development (10).

As a result, E2 allele was in lower syntax score group versus E3 (P=0.046) and E4 (P=0.003) allele; and E4 allele was in higher syntax score group versus E3 alleles (P= 0.034). The Syntax score was seemed to be lower in E2 allele group versus E4 and E3 (P=0.013). As reported in previous studies, E4 allele was related to more extensity of CAD in patients with SHD and E2 was less. Effects of these alleles' contribution to development of CAD need further study.

Limitations

Study was carried out with low number of subjects

CONCLUSION

In conclusion, we reported the first study that patients with SHD, E2 allele is related to less, and E4 allele is related to more severity and extensity of CAD. This conclusion was independent of serum lipid levels which suggesting the APOE gene play role on development of CAD through not only cholesterol uptake but allele-specific antioxidant and immune activities.

Conflict of interest : The authors declare that they have no competing interest.

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Ethical approval: The study was granted ethical approval by the Ethics Committee of Kanuni Training Research And Education Hospital, (Date: 30/12/2015, Decision Number: 23618724).

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