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Effect of Apolipoprotein E Genotypes on Incidence and Development of Coronary Stenosis in Iranian Patients With Coronary Artery Disease

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Background: Apolipoprotein (apo) E polymorphism plays a significant role in the development of coronary disease, but their involvement in coronary artery stenosis (CAS) is controversial. Therefore, the purpose of this study was to investigate the effects of this polymorphism on atherosclerosis, and severity and extent of CAS in unrelated Iranian population. *Methods*: DNA was isolated from 390 study participants and APOE genotypes were determined utilizing the polymerase chain reaction and restriction fragment length polymorphism. *Results*: The APOE-ε4 and -ε2 allele frequencies were significantly higher in the CAS patients than in the control group ($P<0.05$). The association of Apo E polymorphism with the severity of stenosis was evaluated, which is according to the result that apolipoprotein E alleles were not significantly different when compared with the severity of stenosis ($\chi^2 = 0.84$, $P>0.05$). *Conclusion*: Our results suggest that APOE-ε4 is a risk factor for stenosis but does not has any effect on the severity of this disease. J. Clin. Lab. Anal. 25:43–46, 2011. © 2011 Wiley-Liss, Inc.

Key words: Apo E polymorphism; coronary artery stenosis; polymerase chain reaction; restriction fragment length polymorphism

Apolipoprotein E (apoE, protein; APOE, gene) is one of the most studied candidate genes in relation to coronary artery disease (CAD). Plasma apo E is synthesized mainly in the liver and is a constituent of chylomicrons, very low-density lipoproteins, and high-density lipoproteins (HDL) (1). Apo E serves as a ligand for the apo B, E receptor, and the LDL-receptor related protein (2–4), and thus it plays a prominent role in the transportation and redistribution in both the influx and efflux of cholesterol in the body (5). Polymorphisms involving single amino substitution results in three major alleles (ε2, ε3, and ε4) and their six corresponding phenotypes (E2/E2, E3/E3, E4/E4, E2/E3, E2/E4, and E3/E4) (6). The genetic variations at Apo E have been shown to affect on lipid and lipoprotein levels in the general population (7). The ε_4 isoform is associated with increased levels of total cholesterol (TC) and beta lipoprotein and increased susceptibility to CAD (8). In contrast, the E2 isoform exerts opposite effects on blood lipid profile and lipoprotein to those of apo E4 (6,9). Most of the published APOE-CAD association studies have utilized clinically assessed case–control cohorts with limited or no data on the relationship between the APOE polymorphism and angiographic stenosis severity

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as well as the number of affected vessels. In this study, we have examined the association of APOE polymorphism with coronary atherosclerosis and the severity of angiographic CAD in Iranian population.

The study group consisted of 190 patients (140 males and 50 females; age range: 49–70 years) who were admitted to the cardiology unit of Shahid Rajaee hospital of Tehran and Shahid Madani hospital of Tabriz who had been diagnosed to have atherosclerosis. The diagnosis was based on the complete physical and clinical examination of patients by the cardiologist. The patient subjects severity of CAD is determined by a number of coronary arteries as patient with single vessel disease, double vessel disease, and triple vessel disease. For this study, only patients with atherosclerosis were included, whereas patients with Alzheimer disease, pulmonary, renal, hepatic disease, cardiomyopathy congestive heart failure, and acute myocardial infarction were excluded. Healthy individuals were included in the study as controls (100 males, 100 females; age range: 36–62 years). Control subjects were also similarly evaluated for the confounding risk factors included smoking and alcohol consumption, dislipidemia, and family history of atherosclerosis. This study was approved by the University Hospital Ethics Committee and a written informed consent was obtained from all patients and control subjects.

Five millilter of 14-hr fasting venous blood samples were collected from 190 patients and 200 controls. Plasma TC and triglycerides were measured by using routine enzymatic methods. HDL cholesterol was determined after the precipitation of the apoB-containing lipoproteins. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula (10).

Leukocytes were extracted following the standard protocols (11). DNA was amplified by polymerase chain reaction (PCR) in a DNA cycler (0005.416model T-cy grady, Biometra, Goettingen, Germany) using oligonucleotide primer forward (5′-ACAGAATTCG-CCCCGGCCTGGTACAC-3) and reverse (5′-TAAGC-TTGCCACGGCTGCCAAGCA-3′), as described by Hixson et al. (12). The PCR condition included an initial step of 95°C for 30 min, followed by 33 cycles (95°C for 30 sec, 55°C for 30 sec, and 70°C for 1 min) and by a final extension (70°C for 7 min) with 4°C hold. Electrophoresis of amplified products (244 bp) was performed on 10% polyacrylamide gel. After PCR implication, 5 units of Hha1 enzyme (New England Biolabs, Ipswich, MA) were added directly to each reaction mixture for digestion of Apo E sequence of PCR product (over night, 37°C) (12). Each reaction mixture was loaded onto a 10% polyacrylamide gel and electrophoresed. After electrophoresis, digested fragments were visualized by UV illumination. The size of Hha I fragments were estimated by comparison with known DNA (Gene Ruler 50bp DNA Ladder; Fermenta).

T-test was used for comparison of age, BMI, and lipid profile in control and patient groups. Genotype frequencies were compared by the χ^2 test. In order to estimate the risk of coronary artery stenosis, odd ratios were calculated by multiple logistic regression analysis after age and sex adjustments. Allele frequencies were determined by the gene counting method, and Hardy–Weinberg equilibrium was tested by the χ^2 test. The relation between Apo E genotypes and severity of disease in patient group was evaluated by the χ^2 test. Statistical significance was considered significant at the $P<0.05$ levels. All calculations were analyzed with SPSS v.15 software (SPSS for Windows, Chicago, IL).

In this study, we identified three Apo E alleles ε_2, ε_3, and ε_4 and six genotypes $\varepsilon_2/\varepsilon_2$, $\varepsilon_3/\varepsilon_3$ $\varepsilon_4/\varepsilon_4$, $\varepsilon_2/\varepsilon_3$, $\varepsilon_2/\varepsilon_4$, and $\varepsilon_3/\varepsilon_4$. In study population for both men and women, allele frequencies did not deviate from Hardy–Weinberg equilibrium. Statistically significant mean ages, sex, and LDL-C differences were observed between patients and controls ($P<0.05$, Table not shown). The distribution of Apo E genotypes and alleles in patient subjects differed significantly from control group (Table 1). It was observed that the prevalence of $\varepsilon_2/\varepsilon_2$ and $\varepsilon_3/\varepsilon_4$ was not significantly high in patient subjects when compared with controls, whereas the prevalence of $\varepsilon_4/\varepsilon_4$ and $\varepsilon_2/\varepsilon_4$ genotypes was higher in patients than in controls. The frequency of $\varepsilon_2/\varepsilon_3$ and $\varepsilon_3/\varepsilon_3$ in control almost was 1.5- to 2-fold high when compared with patient subjects. APOE-ε4 and APOE-ε2 allele frequencies were significantly higher than those of the control groups ($P<0.05$). Our result also shown that there is no statistically significant difference in association of Apo E alleles and severity of disease ($\chi^2=0.84$, $P>0.05$, Table not shown).

The present case–control study is the first to investigate the association of Apo ε gene polymorphism

TABLE 1. Distributions of Apo E Genotypes and Alleles in Patients and Controls

	Patient $n=190$ (%)	Control $n=200$ (%)	χ^2	*P*-value	OR
Genotype					
$\varepsilon_3/\varepsilon_3$	35(18.42)	59(29.5)	6.53	0.011	0.53
$\varepsilon_4/\varepsilon_4$	34(17.90)	15(7.5)	9.58	0.002	2.70
$\varepsilon_3/\varepsilon_4$	61(32.10)	78(39)	2.019	0.15	0.73
$\varepsilon_2/\varepsilon_3$	10(5.26)	22(11)	4.25	0.039	0.44
$\varepsilon_2/\varepsilon_2$	13(6.85)	7(3.5)	2.23	0.13	2.03
$\varepsilon_2/\varepsilon_4$	37(19.47)	19(9.5)	7.88	0.005	2.31
Alleles					
ε_3	141(37.10)	218(54.50)	$P<0.05$	–	–
ε_4	166(43.69)	127(31.75)	$P<0.05$	–	–
ε_2	73(19.21)	55(13.75)	$P<0.05$	–	–

with CAD in an Iranian population. The frequencies of the different alleles of APOE vary between populations (13). The ε_3 is the most common form of the gene in most of the population (14). In a population-based study, Venkataramana et al. (15) reported that the allele frequencies in Indian population are 85–92% for ε_3 allele, 3.9% for ε_4 allele, and 3.5% for ε_2 allele. In this study, Apo ε allele frequencies in the patient group of Tehran and Tabriz population are 37.10, 43.69, and 19.21% for ε_3, ε_4, and ε_2, respectively, which are not comparable with the study of Venkataramana et al. (15). The reasons for these discrepancies could be genetic heterogeneity and gene environment interactions in different ethnic populations. In this study, we evaluated the distribution of Apo E genotypes and alleles in angiographically defined CAD patients and control subjects, and found these polymorphisms as risk factors for atherosclerosis. As it shown, the distribution of $\varepsilon_4/\varepsilon_4$, $\varepsilon_2/\varepsilon_3$, $\varepsilon_3/\varepsilon_3$, $\varepsilon_2/\varepsilon_4$ genotypes and ε_2, ε_3, ε_4 alleles in patients group was significantly different from control group. It is suggested that the ε_4 allele and $\varepsilon_2/\varepsilon_3$ genotype of Apo E may be less efficient at retarding the oxidation of LDL than others. As it shown in Table 1, the prevalence of $\varepsilon_4/\varepsilon_4$ and $\varepsilon_2/\varepsilon_4$ genotypes was 2- to 2.5-fold high in patient group than in control subjects. This finding suggests that the prevalence of $\varepsilon_4/\varepsilon_4$ and $\varepsilon_2/\varepsilon_4$ genotypes may be risk factors in this complex disease. The frequency of ε_3 allele was 1.5-fold high in control group when compared with patient (54.50 vs. 37.10%, $P<0.05$), while statistically difference was found between patients and controls with respect to ε_2 and ε_4 allele frequencies ($P<0.05$). The strong association of the APOE*4 allele and increased CAD risk has been confirmed in this study. These results showed an evidence of an association between the ε_4 allele and CAD. This finding is in accordance with or different from some studies that were performed in different populations with CAD. These findings are in accordance with the results of two meta-analyses (16). The results of our study with respect to ε_4 allele carriers are in accordance with two meta-analysis studies results. In the general population, the APOE*4 allele is associated with elevated levels of total and LDL-C when compared with APOE*3, while the reverse is true for APOE*2 (9,17). It has been suggested that the presence of APOE*4 allele may lead to an increased level of LDL-C because of the enhanced uptake of apoE4-containing chylomicron remnants and consequently downregulation of the LDL receptor, whereas the APOE*2 allele results in a decreased level of LDL-C level because its lower receptor binding ability delays the removal of chylomicron remnants and upregulation of LDL receptor activity (18). Examining patient files made it clear that these patients used lipid and blood pressure lowering medicines and it seems that the reason for the decrease in LDL-C level and other related lipid profiles is as a result of the use of such medications in this group. In conclusion, our results support the notion that a significant association of ε_4 allele is observed with CAD in addition to the other well-known risk factors and positive family history. Further, this observation interplay between genetic and environmental factors must be thoroughly considered in order to evaluate the etiological role of Apo E in CAD.

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