The Effect of Apolipoprotein E Polymorphism on Plasma Lipoprotein-Lipid Levels in Cardiovascular Disease

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Abstract Apolipoprotein E plays a central role in lipid metabolism by serving as a ligand for the binding of lipoproteins to lipoprotein receptors. We investigated the polymorphism of the apolipoprotein E (ApoE) gene using a PCR-RFLP method and the relation between ApoE polymorphism and plasma lipoprotein-lipid levels in patients with cardiovascular disease (CD). As a result, there were significant differences between plasma lipid profiles in allelic groups of the patients although the frequency of ε4 allele in CD patients was higher than control subject. These results and other recent observation present that one or more factors other than the ApoE gene contribute to the pathogenesis of cardiovascular disease.

Running title: Relationship between ApoE and Cardiovascular Disease

Keywords: Apolipoprotein, Apolipoprotein E, Cardiovascular Disease, Genetic Risk Factors, PCR-RFLP

Introduction

Apolipoprotein E (ApoE) is a protein, which is associated with plasma proteins involved in cholesterol and metabolism. The APOE gene is located on chromosome 19 and includes several alleles. The most common alleles are ε2, ε3 and ε4 corresponding to three isoforms of the apoE protein (1,2,3). The polymorphism is based on the amino acid differences at residues 112 and 158 of polypeptide chain. E3 allele is called as “wild type” since this allele is the most observed one (77%) (1). ApoE polymorphism is one of the common genetic factors responsible for the differences in plasma lipid and lipoprotein levels in human beings (4,5). ApoE polymorphism is considered as a risk factor for coronary artery disease, Alzheimer’s disease, type III hyperlipoproteinemia and possibly vascular dementia (6,7,8). Especially the ε4 allele has been shown that it has an increasing effect on total cholesterol and low-density lipoprotein cholesterol (LDL-C) (8). In spite of these findings, the relationship between apoE alleles might be involved in the pathogenesis of type V hyperlipoproteinemia (9). Individuals with the ε4 allele have total plasma cholesterol levels as well as higher LDL-C levels, and an increasing risk of developing coronary artery disease (10, 11, 12). However, some previous studies indicated that there was no significant relationship between healthy humans and patients with cardiovascular disease while a trend toward higher total and LDL-C from E2 to E4 phenotype was observed (2, 13).

In this study, we analyzed the relationship between apoE phenotype and plasma cholesterol and lipoprotein profiles in healthy human and cardiovascular disease groups.
Materials and Methods

2. 1. Patients
40 patients, all who suffer from cardiovascular disease, (20 men and 20 women) were selected from the biochemical clinic of the State Hospital in Çanakkale (mean age 39±5). Plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), very-low-density lipoprotein cholesterol (VLDL-C) and triglyceride (TG) levels of these patients and, 40 age matched healthy control subject were measured by standardized methods.

2. 2. DNA isolations
High-molecular-weight DNA was obtained from individual blood samples by the genomic DNA isolation kit (MBI Fermentas).

2. 3. Gene Amplification
Enzymatic amplification was performed by primers flanking the apoE locus region which contains the polymorphic sites. The primer sets for the PCR included direct primer (5'-ACAGAATTCGCCCGCTGGTGACAC-3') and reverse primer (5'-TAAGCTTGGCACGGCTGTCCAAGGA-3') (13). The size of the amplified fragment is 244 bp long. Conditions for denaturation, annealing and extension for 30 cycles were 94°C for 40 seconds, 62°C for 40 seconds, 72°C for 60 seconds, respectively and a final extension at 72°C for 5 minutes.

2. 4. Restriction Fragment Length Polymorphism (RFLP)
After purification of 100 μl amplification mix, the resulting PCR was digested 20 units HhaI at 37°C for overnight (14). The restriction fragment were run on 14% polyacrylamide gel (19:1/acr:bis) and analyzed after staining with ethidium bromide.

Results
The plasma lipid profiles and APOE allele frequencies of the subjects are given Table 1. The frequencies observed in all subject are in good agreement with the Hardy-Weinberg equilibrium. As shown in the table, while the ε3 allele frequency of patient group is lower (0,14 in patient group; 0,36 in control), the ε4 allele frequency of patient group is higher (0,43 in patient group; 0,14 control). However, the plasma lipid profiles in cardiovascular patients do not show the differences according to the allele distribution of the APOE gene. In all of three allele types of patients group, plasma levels of TC, HDL-C, LDL-C, VLDL-C and TG are similar to one another. Generally, TC and LDL-C levels of patient group are significantly higher than control subjects. In all control subjects the mean of TC level is 163 mg/dl, the mean of LDL-C level is 91 mg/dl, while the means of TC and LDL-C levels in the patient group are 240 and 165 mg/dl, respectively.

Discussions
According to the previous studies, the relationship between apoE polymorphism and cardiovascular disease is not clear. Our results suggest that there is not a clear relation between two phenomena. There may be other risk factors that are different from apoE polymorphism, such as some genetic factor, feeding, exercises and microbiological or pathological agents. ApoE alleles may have modulating effect on the other risk factors (15). Previous studies in diabetic or nondiabetic cardiovascular diseases and Alzheimer’s disease indicated that some genetic factors could play important roles on the differences of the plasma lipid profiles together with apoE polymorphism in the patients of these disorders (12, 16, 17). Another study has also shown that smoking, drinking and exercising could modulate the effect of ApoE alleles on the plasma lipid profiles (18, 19).

Recently, it is considered that some microorganisms have a significant role in the development of acute myocardial infarction (20, 21). Chlamydia pneumoniae can be given as an important example of microorganisms (22, 23). It is observed that the level of IgG for C. pneumoniae in the patients with acute myocardial infarction and chronic heart disease is higher than control subject. C. pneumoniae infection has a role in the pathogenesis of the cardiovascular diseases. Therefore, hypercholesterolemia (especially LDL-C) is also one of the important risk factors for the myocardial infarction and previous population studies observed that there
was a trend toward higher total and LDL-C from E2 to E4 phenotype (2, 12). That is why the investigation on the relationship between apoE polymorphism and \textit{C. pneumoniae} may be helpful in understanding the development of atherosclerotic mechanisms for further studies.

In conclusion, apoE polymorphism does not influence the occurrence of cardiovascular disease in the group of Turkish patients; therefore, cardiovascular patients with \( \varepsilon 4 \) allele cannot be assumed as an increasing risk of atherosclerotic disease development. Additional studies are necessary to fully clarify the existence of population differences in the influence of apoE polymorphism on the prevalence of cardiovascular disease in those subjects and to determine whether diet and \textit{Chlamydia pneumoniae} could be a confounding factors or not.

### Declaration of Conflicting Interest

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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**Table 1:** Total cholesterol (TC), HDL-C, LDL-C, LDL-C and triglyceride (TG) levels according to the APOE allele types in patients with cardiovascular disease and healthy control subjects.
References


