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Different effects of PPARA, PPARG and ApoE SNPs on serum lipids in patients with coronary heart disease based on the presence of diabetes

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ABSTRACT

Background: The aim of this study was to investigate the individual or combined effects of *PPARA*-L162V, *PPARG*-C161T and *APOE* polymorphisms on hyperlipidemia in coronary heart disease (CHD) patients.
Methods: Our study included 223 patients with CHD (103 with type 2 diabetes (T2DM), 120 without diabetes) and 101 controls. All genotypes were determined by PCR–RFLP technique.
Results: Genotypic and allelic distributions of *PPARA*-L162V polymorphism were similar between study and control groups ($p > 0.05$). The serum total-cholesterol (TC) and LDL-cholesterol (LDL-C) levels were higher in *PPARA*-V162 allele carriers in non-diabetic CHD patients ($p = 0.007$ and $p = 0.038$, respectively). The increasing effect of the *PPARA*-V162 allele on serum TC and LDL-C levels was weakened with the presence of *PPARG-161T* allele in the non-diabetic CHD patients. The *ApoE4–PPARA-V162* allelic combination of the *ApoE/PPARA* genes was found to be more frequent in diabetic CHD patients independent of serum lipids ($p = 0.035$).
Conclusions: The *PPARA* V162 allele has an increasing effect on TC and LDL-C levels and this effect was reduced by carrying PPARG T161 allele in non-diabetic CHD patients. On the other hand, the V162 allele may be associated with an increased risk of CHD in diabetic CHD patients due to the presence of ApoE4 allele independent of serum lipids. We suggest that the *PPARA* L162V polymorphism may have diverse effects on serum lipids and CHD risk depends on the presence of T2DM.

1. Introduction

It is clearly acknowledged that coronary heart disease (CHD) has a multifactorial aetiology, with multiple susceptibility genes interacting with environmental insults to the development of atherosclerosis (National Cholesterol Education Program, 1994). The sequence variations in genes of Apolipoprotein E (*APOE*), peroxisome proliferator activated receptor-alpha (*PPARA*) and -gamma (*PPARG*) have been shown to play roles in hyperlipidemia and atherosclerosis-related diseases (Beaven and Tontonoz, 2006; Garenc et al., 2004; Kisfali et al., 2010).

APOE is a structural component of TG-rich lipoproteins and serves as a ligand for lipoprotein receptors and it mediates the catabolism of chylomicron and very low-density lipoprotein remnants via remnant and low-density lipoprotein (LDL) receptors (Do et al., 2009). A common polymorphism of the *APOE* gene, which encodes human *APOE* protein, results in three major isoforms, *ApoE2*, *ApoE3*, and *ApoE4*, that are coded by E2, E3 and E4 codominant alleles. Among these 3 common *APOE* isoforms, *ApoE4* (Cys112Arg) and *ApoE2* (Arg158Cys) differ from the commonest isoform, *ApoE3*, by a single amino acid substitution (Davignon et al., 1988). Population studies have consistently demonstrated an association between *APOE* and plasma concentrations of total-cholesterol (TC), LDL-C and *ApoB* (Bennet et al., 2007; Davignon et al., 1988). The *Apo*4 allele has been shown to be associated with increased plasma cholesterol levels and increased risk of CHD. In contrast, the *ApoE*2 allele is associated with low plasma concentrations of cholesterol and is believed to be protective against CHD. The major mechanism by which *ApoE*4 increases the risk of CHD may be related to cholesterol elevation (Lahoz et al., 2001).

Abbreviations: PPARA, peroxisome proliferator activated receptor-alpha; PPARG, peroxisome proliferator activated receptor-gamma; APOE, apolipoprotein e; CHD, coronary heart disease; TC, total-cholesterol; TG, triglyceride; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; VLDL-C, VLDL-cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; DM(+)CHD, CHD patients with diabetes; DM(−)CHD, CHD patients without diabetes.

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The peroxisome proliferator-activated receptors (PPARs), a member of the nuclear receptor superfamily, are ligand-dependent nuclear transcription factors with 3 subtypes expressed in humans which are encoded by different genes (*PPARA*, *PPARG*, and *PPARB/D*) (Kota et al., 2005; Wang et al., 1999). The genes regulated by *PPARA* participate in the regulation of key proteins involved in extracellular lipid metabolism, fatty acid oxidation, lipoprotein and glucose homeostasis, and inflammation. Therefore, *PPARA* is a candidate gene whose expression or activity may influence CHD risk through multiple pathways including alterations in lipid or glucose concentrations, obesity, insulin resistance, or the inflammatory response (Do et al., 2009).

Recent studies have revealed the importance of *PPARG* in the physiopathology of atherosclerosis (Wang et al., 1999; Yilmaz-Aydogan et al., 2011). *PPARG* takes part in the regulation of many target gene expressions which involve adipocyte differentiation, lipid metabolism, and glucose homeostasis (Marx et al., 1998). *PPARG* may also act directly on local vascular walls in promoting foam cell formation (Ricote et al., 1998). In view of the particular relevance of the *PPARG* to lipid metabolism and foam cell formation, it is necessary to assess the role of *PPARG* in atherosclerosis.

However, since PPARA and PPARG are nuclear proteins, it is difficult to measure protein levels in vivo, particularly within the local vascular environment. Hence, functional DNA variants in the *PPARA* and *PPARG* genes could provide an indirect assessment of local effect (Do et al., 2009; Peng et al., 2003; Ricote et al., 1998). Several polymorphisms of the human *PPARA* gene have been described. Of these, a C → G transversion at position 484 in exon 5 leads to a substitution of valine for leucine at codon 162 (Do et al., 2009). This missense mutation of leucine 162 to valine (L162V) has functional consequences on *PPARA* activity (Flavell et al., 2002). Previous studies have shown the rare V162 allele association with higher concentrations of TC, LDL-C, apolipoprotein B (ApoB), apolipoprotein C-III (ApoC-III), and triglyceride (TG) (Robitaille et al., 2004; Tai et al., 2006). However, the effects of this polymorphism on plasma lipids and atherosclerosis development are still contradictory (Gouni-Berthold et al., 2004). Also, different mutations have been identified in the *PPARG* gene. The C161T substitution at exon 6 of the *PPARG* gene was reported to be associated with reduced CHD risk. One of the mechanisms may be related to its effect on lipid metabolism (Peng et al., 2003; Wan et al., 2010). In our previous study, we suggested that the C161T polymorphism of the *PPARG* gene was associated with CHD in the Turkish population. Also, we reported that this polymorphism at *PPARG*-locus had deleterious effects on plasma TG levels and therefore may be a potential risk for CHD, especially in patients with diabetes. Furthermore, it was observed that the increasing effects of the C161T polymorphism on serum TG levels could be modified by *PPARG* Pro12Ala polymorphism (Yilmaz-Aydogan et al., 2011).

There are numerous studies regarding the association of PPARA, PPARG and ApoE polymorphisms with the atherosclerosis, type 2 diabetes (T2DM) and insulin resistance (Bennet et al., 2007; Do et al., 2009; Garenc et al., 2004; Lahoz et al., 2001; Peng et al., 2003; Ricote et al., 1998; Robitaille et al., 2004; Tai et al., 2006; Wang et al., 1999). But the combined effects of the genetic variations of these genes on serum atherogenic lipid profile and CHD risk were not analysed comparatively as they depend on the presence of T2DM.

Therefore, the main purpose of this study was to investigate both the independent and combined effects of the common polymorphisms of the *PPARA*, *PPARG* and *ApoE* genes with serum lipids and the risk of CHD and type 2 diabetes on a Turkish population.

2. Materials and methods

2.1. Patient selection and clinical investigation

A total of 223 patients diagnosed with CHD (103 patients with diabetes, 120 patients without type 2 diabetes) at Marmara University, Department of Cardiovascular Surgery, Istanbul, and 101 healthy volunteers as controls were included in this study. None of the diabetic and nondiabetic patients were taking lipid-lowering drug. The patients with severe coronary vascular disease were documented by angiography. The inclusion criteria were, 50% or more stenosis of at least one major coronary vessel with atherosclerotic plaque, or a vascular event which is defined as myocardial infarction or performed percutaneous transluminal coronary angioplasty, or coronary artery by-pass grafting.

Type 2 diabetes was diagnosed according to the American Diabetes Association criteria (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1998) by at least three times repeatedly 6 mmol/L elevated fasting or 11.1 mmol/L elevated 2 h oral glucose tolerance test plasma glucose concentrations. Blood pressure was measured as recommended by the American Association (Kirkendall et al., 1980). The subjects lay supine for 10 min., after which their blood pressure was measured with a mercury sphygmomanometer. The readings were taken from the left and the right arms and recorded to the nearest 2 mm Hg and the mean was calculated. Hypertension was established if the blood pressure was >140/90 mm Hg or if patients were treated with antihypertensive agents.

Healthy subjects (45 (44.55%) women, 56 (55.44%) men), who lack any symptoms of CHD or history of related events, were selected for the control group. Coronary angiography was not performed on these healthy individuals, and therefore the presence of early atherosclerotic coronary artery lesions could not be excluded. All of the individuals of the control group were normotensive and non-diabetic.

The study protocol was approved by both the Ethical Committee of the Istanbul Faculty of Medicine and the Research Fund of Istanbul University. All participants in the study signed informed consent forms in accordance with ethics guidelines regarding the study.

2.2. Lipid measurement

Blood samples were drawn into plain tubes after the participants had fasted overnight. The samples were centrifuged for 10 min at 1500 ×*g* at room temperature and the serum was immediately removed and frozen at −20 °C. The glycerol phosphate oxidase-peroxidase-aminoantipyrine (GPO-PAP) enzymatic calorimetric test was used to measure serum TG levels. The serum TC levels were measured by cholesterol oxidase-peroxidase-aminoantipyrine (CHOD-PAP) enzymatic calorimetric test. Serum HDL-C levels were measured by CHOD-PAP test following the precipitation of apolipoprotein B-containing lipoproteins. Serum LDL-C levels were calculated using the Friedewald formula.

2.3. Genotype study

Genomic DNA was extracted from human leukocyte nuclei isolated from the whole blood by salting-out methods. The *PPARA*-L162V (*rs*1800206), *PPARG*-C161T (*rs*3856806) and *ApoE* (rs7412) polymorphisms were detected by PCR–RFLP analysis as previously reported (Flavell et al., 2002; Kontula et al., 1990; Wang et al., 1999). Real-time PCR were used to confirm the results of *PPARA-L162V*, *PPARG-C161T* and *APOE* polymorphisms for a subset of 20 representative samples, respectively. All results were 100% in concordance with the genotypes determined by conventional PCR–RFLP.

2.4. Statistical analyses

Statistical analysis was performed by using SPSS software package programme (revision 11.5 SPSS Inc., Chicago, IL, USA). Clinical laboratory data are expressed as mean ± SD. Mean values were compared between patients and controls by unpaired Student's *t*-test. Differences in the distribution of genotypes and alleles between cases and controls were tested using the Chi-square statistic. Allele frequencies