Effect of Apolipoprotein E Polymorphism and Apolipoprotein A-1 Gene Promoter Polymorphism on Lipid Parameters and Premature Coronary Artery Disease

(apolipoprotein E gene polymorphism / apolipoprotein A-1 gene promoter polymorphism / familial defective apolipoprotein B-100 / cholesterol / triglycerides / premature coronary artery disease)

D. PETROVIČ[1,2], M. ZORC[2], B. PETERLIN[1]

[1]Division of Medical Genetics, Department of Obstetrics and Gynecology, Medical Center Ljubljana, Slovenia
[2]Institute of Histology and Embryology, Medical Faculty Ljubljana, Slovenia

Abstract. Genetic and environmental factors regulate lipid metabolism and phenotypic expression of CAD. In this study we assessed the effects of apoE gene polymorphism and apoA1 gene promoter polymorphism on lipid metabolism and risk for CAD. In a case-control study, 166 patients with CAD were compared with 130 healthy subjects. The apoE allele frequencies of patients vs. control group were 6.3% vs. 7.7% for e2, 84.3% vs. 84.6% for e3, and 9.4% vs. 7.7% for e4. Individuals with e3e4 and e4e4 genotypes had higher total (P = 0.023) and LDL cholesterol levels (P = 0.04) than individuals with other genotypes. There were no differences in lipid parameters between the subjects with the apoA1-GG genotype and subjects with AG or AA genotypes. However, univariate analysis revealed no association between risk genotypes (e3e4 and e4e4 genotypes) of apoE and CAD risk (OR = 1.1; 95% CI = 0.6-2.1, P = 0.8) as well as no association between the GG genotype and CAD risk (OR 0.7; 95% CI = 0.5-1.2, P = 0.19). No evidence for a synergistic interaction between e3e4 plus e4e4 genotypes and apoA1-GG genotype on CAD risk was found (OR = 1.3, 95% CI = 0.6-2.9; P = 0.5). One individual with familial defective apolipoprotein B-100 (Arg3500Gln) was found in each group.
In conclusion, the apoE gene polymorphism affected the total and LDL cholesterol levels, whereas neither the apoE gene polymorphism nor the apoA-1 gene promoter polymorphism were shown to be independent risk factors for CAD in Slovenia.

Coronary artery disease (CAD) is a multifactorial disorder, influenced by environmental and genetic factors. Large-scale epidemiological studies have led to the identification of a number of the so-called conventional risk factors, such as smoking, arterial hypertension, diabetes mellitus, family history of myocardial infarction (MI), increased serum cholesterol and triglyceride levels, and low serum high-density lipoprotein (HDL) cholesterol level (Gordon et al., 1977; Castelli, 1983; Wilson, 1994; Petrovič et al., 1996). Genetic and environmental factors regulate serum cholesterol and triglyceride levels. Genetic variations of various apolipoproteins have been postulated to be partially involved in regulation of serum total cholesterol, low-density lipoprotein (LDL) cholesterol, HDL cholesterol and triglyceride levels.

Apolipoprotein E (apoE) is a major component of atherogenic triglyceride-rich lipoproteins. Three common variants of apoE have been described (e2, e3, e4) to encode 6 possible phenotypes. A specific interaction between apoE and LDL receptor is an essential mechanism controlling the removal of apoE-rich lipoproteins (very low-density lipoproteins, chylomicron remnants, and intermediate-density lipoproteins), and thus determining the homeostasis of cholesterol and triglycerides (Brown and Goldstein, 1986). Common polymorphism of the apoE gene exerts the most powerful effect on the variability of serum LDL cholesterol level. The e4 allele has been associated with CAD in some but not all studies (Eto et al., 1989; Hixon, 1991; Marshall et al., 1994; Wilson et al., 1994).

Apolipoprotein A-1 (apoA1) is the major protein in HDL and plays a crucial role in lipid metabolism. A single-base variation in the apoA1 gene promoter was reported to influence the response of the serum LDL cholesterol level to dietary fat (Lopez-Miranda et al., 1994) and to be associated with HDL cholesterol in women (Pagani et al., 1990) and in men (Jeenah et al., 1990). Plasma levels of apoA1 and HDL cholesterol levels are inversely correlated with CAD (Gordon et al., 1977).

Apolipoprotein B-100 (apoB100) is the major protein in LDL, which plays an important role in LDL cholesterol metabolism. There are three mutations in the apoB100 gene (Arg3500Gln, Arg3531Cys, and Arg3500Trp). Among these three mutations, only the first, called familial defective apoB100, was shown to be an inde-

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Corresponding author: Danijel Petrovič, Institute of Histology and Embryology, Medical Faculty of Ljubljana, Korytkova 2, 1105 Ljubljana, Slovenia. Tel: +386 61 441 121; Fax: +386 61 1401 294; e-mail: daniel.petrovic@mf.uni-lj.si.

Abbreviations: apoA1 – apolipoprotein A-1, apoB100 – apolipoprotein B-100, apoE – apolipoprotein E, CAD – coronary artery disease, HDL – high-density lipoprotein, LDL – low-density lipoprotein, MI – myocardial infarction, OR – odds ratio, PCR – polymerase chain reaction, WHO – World Health Organization.

pendent risk factor for CAD in the Danish population (Tybjaerg-Hansen et al., 1998).

The aim of this study was to assess the effects of apoE gene polymorphism and apoA1 gene promoter polymorphism on serum cholesterol and triglyceride levels and on the phenotypic expression of CAD. Additionally, we wanted to assess the incidence of apoB100 (Arg3500Gln) mutation in patients with CAD.

Material and Methods

Subjects

The study population consisted of 166 patients with premature CAD (134 with MI and 32 with unstable angina) and 130 healthy subjects. The diagnosis of MI was made according to the criteria of the World Health Organization (WHO, 1985). Patients with MI younger than 55 years were included 1–9 months after the acute event. The patients and control subjects came from independent families and were all Slovene. The research protocol was approved by the national ethics committee. After an informed consent had been obtained from the patients and control subjects, a detailed questionnaire was taken. Each patient was interviewed by the physician about coronary risk factors (diabetes, cigarette smoking, arterial hypertension, body weight and height). The smoking habit was defined as a daily intake of more than 5 cigarettes. Arterial hypertension and diabetes mellitus were defined as binary variables. The diagnosis of diabetes mellitus was made according to the WHO (Chetlin et al., 1993).

Lipid analysis

Total cholesterol, LDL, HDL, and triglycerides were determined by standard chemical methods.

Genetic analysis

The polymerase chain reaction (PCR) for each system (apoE, apoA1 gene promoter, apoB100 mutation) was carried out in a 15-μl reaction volume containing 0.05 mM dNTP (Gibco, Paisley, UK), 10 mM TRIS HCl buffer, 1 mM $MgCl_2$, 0.5 μM of each primer (5'-GCA CGG CTG TCC AAG GAG CTG CAG GC-3', 5'-GGC GCT CGC GGA TGG CGC TGA G-3' for apoE gene polymorphism; 5'-CAC CCG GGA GAC CTG CAA GC-3', 5'-TCT AAG CAG CCA GCT CTT GCA-3' for apoA1 gene promoter polymorphism; 5'-CAC CCG GGA GAC CTG CAA GC-3', 5'-TCT AAG CAG CCA GCT CTT GCA-3' for apoB100 mutation), 500 ng of genomic DNA and 0.5 units of Taq DNA polymerase (Gibco). DNA was amplified for 30 cycles with denaturation at 94°C for 1 min, annealing at 59°C for 1 min, and primer extension at 72°C for 1 min. ApoE gene polymorphism – 8 μl of the PCR product was digested overnight at 37°C with 10 units of *Hha*I, and the fragments were then separated on 15% polyacrylamide gels (Hixon and Vernier; 1990). ApoA1 gene promoter polymorphism – 8 μl of the PCR product was digested overnight at 37°C with 10 units of *Msp*I, the fragments were separated on 2% agarose gels and visualized with ethidium bromide. ApoB100 mutation – 8 μl of the PCR product was digested overnight at 37°C with 10 units of *Msp*I, the fragments were separated on 2% agarose gels and visualized with ethidium bromide.

Statistical methods

The clinical characteristics of the two groups were expressed as mean standard deviation (SD) and were compared by unpaired Student's t test; χ^2 analysis was used to compare discrete variables. An odds ratio (OR) was calculated as a measure of relative risk. Univariate analyses were used in the assessment of the effect of gene polymorphisms on the risk of CAD. Statistical analysis was performed using the SPSS program 9 for Windows (SPSS Inc., Illinois).

Results

The characteristics of the patients and control subjects are listed in Table 1. Ninety percent of the cases were males. A higher incidence of diabetes (10% vs. 4%; $P = 0.024$) and cigarette smoking (65% vs. 35%; $P < 0.001$) was found in cases than in the control group. The cases had a lower HDL cholesterol level (1.1 ± 0.4 vs. 1.2 ± 0.5, $P < 0.001$) than the subjects in the control group. No significant differences were found in age, total cholesterol, LDL cholesterol, triglyceride levels, body mass index (BMI), and incidence of hypertension between the patients and control subjects (Table 1).

The apoE allele frequencies in patients with CAD vs. subjects in the control group are shown in Table 2. There were no statistically significant differences in apoE allele frequencies between the patients with premature CAD and the control group.

Subjects (patients and controls) with e3e4 and e4e4 genotypes had higher total and LDL cholesterol levels than subjects with other apoE genotypes, whereas no statistically significant differences were found in other lipid parameters (Table 3).

Subjects (patients and controls) with the GG genotype of the apoA1 gene promoter polymorphism did not have lower serum HDL cholesterol levels than the subjects with AG or AA genotypes (1.16 ± 0.51 mmol/l vs. 1.15 ± 0.51 mmol/l; $P = 0.91$) and the polymorphism did not affect the total cholesterol (6.41 ± 1.62 mmol/l vs. 6.64 ± 1.7 mmol/l; $P = 0.25$), LDL cholesterol (4.28 ± 1.55 mmol/l vs. 4.31 ± 1.57 mmol/l; $P = 0.89$), and triglyceride levels (2.15 ± 1.44 mmol/l vs. 2.39 ± 1.69 mmol/l). With regard to HDL cholesterol, smokers had a lower serum HDL cholesterol level than non-smokers (1.11 ± 0.51 mmol/l vs. 1.23 ± 0.50 mmol/l; $P = 0.04$). When we wanted to assess the effect of genotypes in smokers only, no difference between the GG genotype and AG or AA