# GENETICS

# Apolipoprotein E Gene Polymorphism in Men with Coronary Atherosclerosis in Siberia

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 150, No. 9, pp. 324-327, September, 2010
Original article submitted September 21, 2009

Polymorphism of coding fragment of *APOE* gene was analyzed in two groups of men. The main group consisted of 77 residents of the West Siberian region aged 45-65 years with coronary atherosclerosis (documented by coronary angiography) without acute coronary syndrome with stable effort angina, functional class II-IV. The reference group consisted of 350 residents of Novosibirsk, aged 45-69 years. Statistically significant associations between genotypes of *APOE* gene coding part polymorphism and some key lipid risk factors (blood total and LDL cholesterol, atherogenic index, *etc.*) for coronary atherosclerosis were found in male residents of the West Siberian region. Elevated total mean level of cholesterol was detected in male residents of Novosibirsk with the *APOE* genotypes containing ε4 allele.

**Key Words:** *coronary atherosclerosis; population; polymorphism; apolipoprotein E gene; blood lipid profile*

Coronary atherosclerosis is a highly prevalent disease in the majority of world populations. Detection of genetic markers of liability to the disease and studies of their specific features in different populations can become effective methods for its early diagnosis and prevention.

Many gene polymorphisms presumably associated with atherosclerosis have been studied, including LDL receptor gene [1] and apolipoprotein (apo) B gene polymorphisms [13], but the best studied is polymorphism of encoding part of apo-E (*APOE*) gene, caused by the presence in a special locus of three alleles – ε2, ε3, and ε4, inherited by the co-dominant mode. The apo-E protein is presented by several isoforms: apo-E2, apo-E3, and apo-E4. The incidence of *APOE* gene alleles varies in the world populations. The ε3 allele is the most prevalent, its incidence being 0.60-0.88; the incidence of ε2 and ε4 varies greatly [4,6,9,12].

The *APOE* polymorphism is associated with blood cholesterol (CH) level and with liability to coronary disease [11,15]. The presence of ε4 allele is often linked with high level of CH-rich LDL particles in the blood. This is due to the fact that, first, apo-E is the key ligand of LDL particles for apo-B,E receptors and, second, it is involved in the formation of LDL particles in the blood from intermediate density precursor lipoproteins (IDL). The clearance of remnant lipoproteins containing apo-E2 is very slow because of ineffective apo-E2 binding to apo-B,E receptors; transformation of ILD particles into LDL is also disordered. Reduced delivery of CH into the liver leads to activation of apo-B,E receptor synthesis, enhanced binding of LDL particles by these receptors, and to a

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0007-4888/11/1503-0355 

decrease in LDL concentration in the blood. By contrast, clearance of apo-E4-carrying particles is rapid, which leads to reduction of apo-B,E receptor synthesis and elevation of LDL CH level in the blood [14]. The level of CH in the blood in the presence of ε2 allele is 10% lower in comparison with those in the presence of ε3 homozygotes and is 5% higher in the presence of ε4 allele [8]. Allele ε4 is associated with high risk of coronary disease in comparison with ε3 homozygotes, while for subjects with ε2 allele the risk is the minimum [9]. However, not all subjects with apo-E4 phenotype develop hypercholesterolemia [5]. Many factors, including hormonal and alimentary, can compensate for the functional defects of apo-E4 phenotype.

The specificity of the relationship between *APOE* gene coding part polymorphism and atherosclerosis risk factors in different populations and ethnic groups necessitates studies of this problem for populations living in different climatic geographical zones, including residents of West Siberia. This study was carried out within the framework of this problem.

## MATERIALS AND METHODS

The study was carried out in 2 groups of men. The main group (group 1) consisted of 77 patients (residents of West Siberian region) aged 45-65 years with documented (by selective coronary angiography on an Advantex LC/LP angiographic device, General Electric) coronary atherosclerosis without acute coronary syndrome with stable effort angina, functional classes II-IV. Seventy five percent of patients in this group had a history of myocardial infarction 6 months or more before the study. The history of coronary disease varied from 1 to 36 years. The distribution of effort angina by functional classes was as follows: class II in 14.5%, class III in 70%, and class IV in 155% patients. Study of the coronary risk factors in these patients revealed arterial hypertension in 87%, type II diabetes mellitus in 22%, and 43% were tobacco smokers. Mean body weight index in the group was 30.2±2.5 kg/m$^2$.

The reference group (group 2) consisted of 350 men aged 45-69 years, residents of Novosibirsk, examined in 2007-2008 during screening within the framework of HAPIEE International Multi-Center Project (Head Center in London, principal researchers in Novosibirsk Yu. P. Nikitin, Member of the Russian Academy of Medical Sciences, and Professor S. K. Malyutina). Coronary disease was determined in men of the population group by validated epidemiological (including Rows' cardiological questionnaire) and clinical functional methods (ECG record with the Minnesota code deciphering).

The blood was collected from the ulnar vein after overnight (12 h) fasting in men of both groups. Serum lipid profile (total CH, triglycerides, HDL CH and LDL CH) and blood glucose were measured by enzymatic methods using standard Biocon Fluitest reagent kit on a Labsystem FP-901 biochemical analyzer. The atherogenic index was calculated by the formula: (total CH-HDL CH)/HDL CH. Blood apo-A1 and apo-B levels were measured by immunoturbidimetric method using DiaSys standard reagents.

Serum levels of highly sensitive C-reactive protein and C-peptide were measured by EIA with standard ELISA Biometrica test systems on a Multiscan EX analyzer. The indicators of LPO processes activity (initial level of lipid peroxides in LDL evaluated by MDA concentration and LDL resistance to *in vitro* oxidation in the presence of copper ions) were evaluated by fluorometry on a VersaFluor fluorometer (Bio-Rad).

Isolation of DNA from blood and tissues was carried out by modified phenol-chloroform extraction [3].

Analysis of *APOE* gene coding part polymorphism in 3937C/T and 4075C/T positions was carried out. Genotyping of *APOE* gene coding part polymorphism was carried out by a previously described method [7]. The genome DNA was amplified by PCR in standard reaction mixture and then hydrolyzed by AspLE I restrictase with GCGC recognition site. Restriction product visualization was carried out by gel electrophoresis in 10% PAAG with subsequent staining by ethidium bromide and scanning of the gel by computer videorecording.

Statistical analysis was carried out using $\chi^2$ test for evaluating the significance of differences in allele frequencies in the two populations and for testing the Hardy–Weinberg equilibrium. The differences in the mean quantitative values of subjects with different genotypes were evaluated after standardization by gender, age, and body weight index.

## RESULTS

The incidence of genotypes and alleles of *APOE* gene coding part polymorphism was determined and the correspondence of the genotype incidence to the Hardy–Weinberg equilibrium was evaluated by $\chi^2$ test (Table 1).

The ε3 allele was the most incident in both groups. The incidence of alleles and genotypes was close to that observed in the populations of Western and Eastern Europe [6,12]. The incidence of genotypes corresponded to the Hardy–Weinberg equilibrium. No statistically significant differences in the incidence of alleles and genotypes of *APOE* gene coding part polymorphism in the two groups of men were detected.

Associations of *APOE* gene coding part polymorphism with the parameters of blood lipid profiles were analyzed.

The differences between the genotypes in the mean levels of blood total CH were statistically significant ($p$=0.01) in group 2 (Table 2). The maximum mean levels of total CH in group 2 were detected for genotypes containing allele ε4, the minimum ones for ε2/ε2 and ε2/ε3 genotypes. No statistically significant differences in the mean levels of total CH for subjects with different genotypes were found in group 1.

In group 2, the differences in the mean levels of LDL CH in the blood were also statistically significant (Table 2). The maximum mean LDL CH values in group 2 were detected for genotypes ε2/ε4 and ε3/ε4, the minimum values for genotypes ε2/ε2 and ε2/ε3. No statistically significant differences in the mean levels of LDL CH were detected in subjects with different genotypes in group 1.

The results of atherogenic index evaluation were similar in the two groups (Table 2). The absence of the maximum levels of LDL CH and atherogenic index in men with the ε4/ε4 genotype (the most atherogenic genotype) in both groups can be due to a very little number of individuals with this genotype included in the study ($n$=1 and $n$=2, respectively). However, we can see a trend to the maximum level of these parameters in group 1 subjects with genotype ε4/ε4 (Table 2).

The minimum mean HDL CH levels in group 2 were found for genotypes containing allele ε4. No statistically significant association of the *APOE* gene polymorphism and mean HDL CH level was detected in the common factorial model of the studied groups. No independent association of the *APOE* gene coding part polymorphism and mean level of triglycerides in the blood was found in the analyzed groups.

**TABLE 1.** Incidence of *APOE* Gene Coding Part Polymorphism Genotypes and Alleles (in %)

| Parameter | Group 1 ($n$=77) | Group 2 ($n$=350) |
|---|---|---|
| Genotype | | |
| ε2/ε2 | 1.3 ($n$=1) | 0.9 ($n$=3) |
| ε2/ε4 | 2.6 ($n$=2) | 1.1 ($n$=4) |
| ε2/ε3 | 6.5 ($n$=5) | 12.3 ($n$=43) |
| ε3/ε3 | 70.1 ($n$=54) | 64.3 ($n$=225) |
| ε3/ε4 | 18.2 ($n$=14) | 20.9 ($n$=73) |
| ε4/ε4 | 1.3 ($n$=1) | 0.6 ($n$=2) |
| Allele | | |
| ε2 | 5.84 | 7.52 |
| ε3 | 82.47 | 80.86 |
| ε4 | 11.62 | 11.57 |
| $\chi^2$ test | 3.811 | 3.698 |
| Conformity with Hardy—Weinberg equilibrium | 0.238 | 0.98 |

Study of associations of the *APOE* gene (group 1) coding part polymorphism and LDL levels of LPO products and blood glucose levels showed no statistically significant differences. However, an association of *APOE* gene coding part polymorphism with body weight index and mean level of C-peptide in the blood was revealed ($p$=0.034 and $p$=0.000, respectively).

On the other hand, no associations of *APOE* gene coding part polymorphism with body weight index, mean levels of C-peptide, highly sensitive C-reactive protein, apo-A1, apo-B, and glucose in the blood were detected in group 2 men. However, a statistically sig-

**TABLE 2.** Blood Levels of Total CH, LDL CH (mg/dl) and Atherogenic index for Different Genotypes of *APOE* Gene Coding Part Polymorphism ($M\pm\sigma$)

| Parameter | Total CH | | LDL CH | | Atherogenic index | |
|---|---|---|---|---|---|---|
| | group 1 | group 2 | group 1 | group 2 | group 1 | group 2 |
| ε2/ε2 | 208.2±56.0 | 185.5±21.8 | 118.0±47.9 | 91.1±19.3 | 3.4±2.3 | 2.3±0.6 |
| ε2/ε4 | 181.1±38.2 | 215.9±21.9 | 123.1±32.7 | 148.4±19.4 | 5.0±1.6 | 3.7±0.6 |
| ε2/ε3 | 277.6±24.5 | 198.6±6.2 | 193.5±21.0 | 116.4±5.5 | 6.9±1.0 | 2.7±0.2 |
| ε3/ε3 | 231.2±7.5 | 215.4±2.6 | 156.5±6.5 | 136.1±2.3 | 5.5±0.3 | 3.1±0.1 |
| ε3/ε4 | 236.2±15.0 | 226.9±4.7 | 164.7±12.8 | 146.9±4.2 | 6.3±0.6 | 3.4±0.1 |
| ε4/ε4 | 239.4±54.4 | 224.5±37.7 | 169.8±46.6 | 133.6±33.4 | 7.5±2.2 | 2.8±1.0 |
| $p$ | 0.363 | 0.010 | 0.420 | 0.0001 | 0.478 | 0.010 |

**Note.** $p$: level of statistic significance of this factor in the total factorial model.

nificant association of *APOE* gene polymorphism with indicators of LPO product levels in LDL was found in this group, specifically, with the initial level of LPO products in LDL ($p$=0.006) and LDL resistance to oxidation *in vitro* in the presence of copper ions ($p$=0.019).

Hence, we found statistically significant associations between the *APOE* gene coding part polymorphism genotypes and some key lipid risk factors (blood total CH and LDL CH, atherogenic index, *etc.*) for coronary atherosclerosis in men living in West Siberian region. Higher mean levels of total CH in the blood were found for genotypes containing allele ε4 in men from the Novosibirsk population. Associations of this kind between the mean blood lipid levels and the studied polymorphism were previously detected for the population of Novosibirsk aged 25-64 years [2]. The data in general confirm an important contribution of the genetic component to the formation of cardiovascular risk.

The detected associations of the *APOE* gene coding part polymorphism with body weight index and blood level of C-peptide in group 1 confirm the role of non-lipid risk factors (including metabolic risk factors) in the development of coronary atherosclerosis. Normally insulin inhibits lipolysis in the liver. Insulin resistance of tissues prevents inhibition of lipolysis in the liver, and triglycerides and total CH as components of VLDL and LDL are more intensely released into the blood, while HDL CH level decreases, which leads to the development of atherogenic dyslipoproteinemia characteristic of these disorders [10]. Interactions of LDL with cellular apo-B,E receptors, more pronounced in the presence of allele ε4, are modified under conditions of insulin resistance and glucose metabolism disorders. The sum of these factors is responsible for high atherogenic potential of the blood lipid spectrum in group 1 in comparison with group 2. In general, this explains our results indicating a relationship between apo-E gene polymorphism and some disorders in lipid and carbohydrate metabolism.

The detected association of the *APOE* gene coding part polymorphism with lipid and non-lipid risk factors for atherosclerosis development suggests studies of gene polymorphisms and creation of atherosclerosis prevention programs based on the results of the *APOE* gene coding part polymorphism genotyping for evaluation of individual risk of atherosclerosis development.

The study was supported by the Russian Foundation for Basic Research (grant No. 09-04-01387a), President of the Russian Federation (grant No. MK-2666.2009.7), Mayor Administration of Novosibirsk (grant No. 22-08, NIA No. 1 RO1 AG23522-01).

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