Abstract  Familial hypercholesterolaemia is a genetic disorder characterised by high low-density lipoprotein (LDL) cholesterol concentrations, which frequently gives rise to premature coronary artery disease (CAD). The clinical expression of familial hypercholesterolaemia is highly variable even in patients carrying the same LDL receptor gene mutation. This variability may be due to environmental and other genetic factors. Apolipoprotein E (Apo-E) has been extensively studied for its effects on the phenotype of familial hypercholesterolaemia. In this study we examined the influence of Apo-E genotype on lipid parameters and the incidence of CAD in 93 Greek patients with familial hypercholesterolaemia. Apo-E E2, E3 and E4 allele frequencies were 0.06, 0.86 and 0.09 respectively. The levels of total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, apolipoproteins A and B and lipoprotein α did not differ significantly among carriers and non-carriers of the E4 allele. The prevalence of CAD and hypertension did not differ either. Our results suggest that the E4 allele is not associated with lipid levels or with the prevalence of CAD among familial hypercholesterolaemia patients of the Greek population.

Key words  Apolipoprotein E • E4 allele • Familial hypercholesterolaemia • Lipids • Coronary artery disease

Introduction  Familial hypercholesterolaemia (FH) (MIM #143890) is an autosomal dominant disorder caused by mutations which are mainly located in the low-density lipoprotein receptor (LDLR) gene (LDLR; MIM *606945, Genbank Accession Number: NM_000527), or more rarely within the apolipoprotein B-100 (APOB) gene (APOB; MIM +107730, Genbank Accession Number: NM_000384), affecting approximately one in 500 individuals worldwide [1]. It is characterised by a high concentration of low-density lipoprotein (LDL), which frequently gives rise to tendon xanthomas (TX) and premature coronary artery disease (CAD). More than 900 mutations in the LDLR gene have been described and have been associated with FH. In Greece, 16 mutations in the LDLR are responsible for heterozygous FH and among them V408M, G528D, C6W and S265R account for 73% of heterozygous FH probands [2, 3]. The impact of these mutations on the LDL-R function has been characterised by considerable variability and in this regard we have previously shown that the carriers of receptor negative mutations have higher levels of total cholesterol (TC), LDL cholesterol and higher prevalence of TX compared to carriers of receptor defective mutations.
Apolipoprotein E (Apo-E) plays an important role in lipoprotein metabolism as it mediates the elimination of triglyceride (TG)-rich lipoproteins. It serves as a ligand for different receptors including the LDL-R. Structural defects of Apo-E might result in an impaired interaction of Apo-E containing lipoproteins with its receptors and lead to the development of atherogenic dyslipidaemias and premature cardiovascular disease [8].

Natural variants of the Apo-E (ApoE) gene (APOE; MIM +107741, Genbank Accession Number: NM_000041) give rise to the occurrence of three isoforms: E2, E3 and E4. The E2 isoform is functionally inactive and is only associated with premature vascular disease under certain conditions [8]. Compared to E3, the E4 isoform has been associated with higher plasma cholesterol concentrations and an increased risk of coronary heart disease in the general population [9–12]. The effect of ApoE genotype on lipid parameters and CAD risk in FH patients has been assessed by several research teams and the results are conflicting [13–22].

As the effect of ApoE genotype on lipid parameters in FH patients is still controversial, the aim of this study was to investigate the association between the ApoE genotype and plasma lipid levels and its contribution to the prevalence of cardiovascular disease.

**Patients and methods**

Study population

A total of 104 patients (males and females) were investigated. Clinically diagnosed FH patients referred to us for genetic analysis had elevated TC (>290 mg/dl) and LDL-C (>200 mg/dl) above the 95th percentile for age and sex with normal TG levels (<175 mg/dl). The end point of this analysis was a first, fatal or non-fatal coronary heart disease event (according to the World Health Organization – International Coding of Disease) as most had a history of CAD and a family history (among first- or second-degree relatives) of hypercholesterolaemia and CAD. Family history of premature coronary heart disease in first-degree relatives was recorded in all participants (male relative <45 years old, female relative <55 years old). TX were present in some families and absent in others. Secondary causes for hypercholesterolaemia such as hypothyroidism, diabetes, renal or hepatic disease were absent. Smoking habit (SH) was determined from patient notes and subjects defined as a smoker, ex-smoker or not a smoker. Two recent blood pressure measurements were taken into account from patients’ medical records. As it is commonly used in epidemiological studies and following the guidelines provided by the 7th report of the joint national committee on the prevention, detection, evaluation and treatment of high blood pressure, patients whose average blood pressure levels were ≥140/90 mmHg or were under antihypertensive medication were classified as hypertensives. An institutional review committee approved this study, and informed consent for participation was obtained from all subjects.

Biochemical analysis

Blood samples from the antecubital vein were collected between 8 and 10 a.m., in a sitting position after 12–14 h of fasting and avoidance of alcohol. TC, high-density lipoprotein (HDL) cholesterol and TG were measured using a chromatographic enzymatic method using a Technicon automatic analyser RA 1000 and LDL cholesterol was calculated with the Friedewald formula. Apolipoproteins A1 and B were measured by a BNII Dade Behring automatic nephelometer.

Mutation analysis and ApoE genotyping

Genomic DNA was extracted by the salting-out method [23]. ApoE genotyping was performed using a prototypic multilocus genotyping assay focused on cardiovascular diseases essentially as in Cheng et al. [24]. Briefly, each sample is amplified by a 33-cyclo polymerase chain reaction (25 ng of genomic DNA). PCR product was then hybridised to oligonucleotide probes that had been immobilised in a linear array on a backed nylon membrane strip. The colorimetric detection was based on streptavidin-horse-radish peroxidase conjugate and substrates. Denaturing gradient gel electrophoresis (DGGE) analysis was performed as previously described [25]. Automated sequencing of DGGE patterns representing LDLR polymorphisms was carried out using an ABI PRISM DyeDeoxy Terminator Cycle Sequencing Kit with a ABI 3700 Genetic Analyzer (Perkin Elmer, Applied Biosystems, CA, USA) according to the manufacturer’s instructions.

Statistical analysis

A test for Hardy-Weinberg equilibrium was performed using χ²-tests. Continuous variables are presented as mean values±standard deviation (SD), while qualitative variables are presented as absolute and relative frequencies. Statistical analysis was performed using the SPSS 10.0 (SPSS Inc) program. The independent categorical variables were coded as follows: 0 for female and 1 for male sex, 0 and 1 for absence and presence respectively.

The ApoE genotypes were grouped as follows: 1 for 23,33 and 2 for 34,44. Statistically significant differences among groups were evaluated by the Mann-Whitney test due to the small number of cases and the skewed distribution of the lipid values.

The effect of ApoE genotype on lipid values was evaluated by a fixed effect model, where values were adjusted for age, sex and BMI. The results from the regression models are presented as B-coefficients and standard error of the coefficient.