Apolipoprotein E Genotype in Matched Men and Women with Coronary Heart Disease

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Abstract. Apolipoprotein E (apo E) plays an important role in lipid metabolism and its polymorphism may be a risk determinant of coronary heart disease (CHD). Since evidence suggested a gender-specific effect of apo E polymorphism, we studied the influence of gender-specific interaction of the polymorphism on CHD. From a total of 463 Greek Caucasians (314 men and 149 postmenopausal women) with angiographically documented CHD, we selected 79 women (68 ± 9 yr old) and 79 men (66 ± 9 yr old) who were matched for clinical characteristics. Apo E genotyping was performed by PCR and RFLP analysis. Biochemical parameters were also measured. The results were as follows: the E3/3 genotype occurred in 78.5% of the patients, followed by E3/4, E2/3, E2/4, and E4/4 genotypes, which occurred in 9.5%, 9.5%, 1.9%, and 0.6% of the patients, respectively. No significant differences were observed in the apo E allele or apo E genotype distributions between the matched Greek men and women with CHD. The E3/3 men patients were more frequently part of a family with a history of CHD, compared to women (p = 0.035).

Keywords: apolipoprotein E, polymorphism, coronary heart disease, risk stratification

Introduction

Coronary heart disease (CHD) is the leading cause of mortality in developed countries [1]. CHD is often initially detected from clinical manifestations such as myocardial infarction, angina, or sudden death due to artery occlusion. Given the severity of such disease symptoms, research interest has been focused on the identification of asymptomatic individuals with increased risk of coronary atherosclerosis, so that treatment can be provided before they reach the clinical horizon. Taking into account that apolipoprotein E (apo E) plays an important role in lipid metabolism and that hypercholesterolemia is an independent risk factor for premature CHD, apo E may be an important risk determinant for CHD [2,3]. A meta-analysis of 14 studies showed the E4 allele to be associated with CHD in both women and men [4]. Several other studies, including ours, failed to find any association of the E4 allele with either CHD or myocardial infarction [5-7]. In contrast, an inverse association of the E2 allele and myocardial infarction was found [7]. However, in another study, the presence of the E2 allele in men was shown to be associated with a greater risk for cardiovascular disease [8]. The E4 allele has been characterised as a strong independent predictor of coronary events in men, but not in women [9,10]. Other studies also suggested gender-specific effects of apo E polymorphism [11-14]. The inconsistent relationship between cardiovascular disease and apo E polymorphism can be ascribed to gender-, ethnic-, and population-related differences. In order to evaluate the gender-interaction of the apo E polymorphism with the occurrence of CHD...

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disease, men and women with proven CHD and similar clinical characteristics were matched in the present study. No significant differences were found between men and women in regard to apo E genotype distribution in these Greek patients with CHD.

Materials and Methods

Study population. During the first 4 mo of 2002, a total of 463 Greek caucasians (149 women and 314 men) from various parts of Greece were admitted to the Onassis Cardiac Surgery Center for coronary arteriography investigation. Of this population, we initially selected men and women who fulfilled the following criteria:

1. Angiographically documented CHD, diagnosed with stenosis >50% in at least one major coronary artery (using the Judkins technique), was found after coronary catheterization [15]. The CHD was classified as 1-, 2- and 3-vessel disease.

2. Subjects’ lipid profile was not influenced by any hypolipidemic treatment. Patients who were receiving hypolipidemic drugs were included if they had a lipid profile, within 6 mo before admission to the hospital, that was uninfluenced by hypolipidemic treatment.

3. All women were in the postmenopausal status. Women were classified as postmenopausal if their last menses occurred ≥12 mo earlier and they were not receiving hormone replacement therapy.

The groups of CHD men and women were then matched according to age, body mass index, lipid and lipoprotein levels, and CHD-manifestations. The matching was designed to exclude the possible confounding influences of these factors on CHD risk. Thus, 79 women (68 ± 9 yr old) and 79 men (66 ± 9 yr old) were finally selected.

The Onassis Cardiac Surgery Center ethics committee approved the protocol of this study.

Apo E genotyping. The apo E gene, located on the long arm of chromosome 19, is characterised by 3 common alleles (E2, E3, and E4), which are the result of the variation in codons 112 and 158 [16,17]. Specifically, the E3 allele contains the amino acids cysteine and arginine at codons 112 and 158, respectively. The E2 allele differs from E3 by a cysteine for arginine substitution at amino acid residue 158, while the E4 allele has an arginine for cysteine substitution at residue 112. The combination of these alleles results in 6 different genotypes (E2/2, E2/3, E2/4 E3/3, E3/4, and E4/4) [18,19]. After the recruitment of the study population, the apo E genotyping was performed as described in a previous study [7]. Genomic DNA was extracted by standard methods from 5 ml of whole blood drawn on EDTA. Amplification of the apo E gene 244 bp fragment was performed by polymerase chain reaction (PCR) [20]. Amplification products of the apo E gene poly-morphic sequences were digested with restriction endonuclease HhaI at 37°C for 3 hr to ensure full digestion. Four non-polymorphic recognition sequences of HhaI exist within the 244 bp PCR product, yielding 5 constant fragments. The presence or absence of 2 polymorphic HhaI cleavage sites clearly distinguished the 3 major alleles and the 6 homozygous and heterozygous combinations. The 6 genotypes were characterized after 8% non-denaturing polyacrylamide gel electrophoresis and subsequent ethidium bromide staining.

Biochemical analyses. Plasma total cholesterol, triglyceride, and high-density lipoprotein cholesterol concentrations were measured using enzymatic colorimetric methods on a Roche Integra Biochemical analyzer with commercially available kits (Roche Diagnostics, Mannheim, Germany). Plasma low-density lipoprotein cholesterol levels were calculated, using the Friedewald formula, only in patients with triglyceride levels <400 mg/dl (<4.5 mmol/L) [21]. Lipoprotein(a) was measured by nephelometry (BN-100 nephelometer, Behring, Germany). Plasma glucose was measured by the hexokinase method with a Dade Behring reagent on a Dimension analyzer (Dade Behring, Liederbach, Germany). All samples were analysed within 24 hr after blood collection.

Statistical analysis. Continuous variables are presented as mean ± SD, while qualitative variables are presented as absolute and relative frequencies. The direct gene counting method was used to estimate the allele and genotype frequencies of the apo E gene. The statistical evaluation was based on the calculation of the Chi-square test.

Student’s t-test was used to evaluate differences between genders in continuous measurements. Contingency tables were consulted to evaluate the association between apo E polymorphism and gender of the participants, and differences between genders were tested by the Z-test, after correcting for multiple comparisons using the Bonferonni rule.

Based on statistical power estimates, the enrollment of 79 men and 79 women patients was adequate to evaluate >1.0 two-tailed standardised differences of the investigated prevalence of the genotypes between genders, achieving statistical power >0.90 at <0.05 p value. The reported p values are from 2-sided tests at the <5% probability level. STATA 6 software was used for the calculations (STATA Corp, College Station, Texas, USA).

Results

Table 1 describes the clinical characteristics of the male and female CHD patients. Data presented in Table 1 document the matching procedure applied for the selection of patients. In particular, no significant differences were observed between the genders in regard to age, body mass index, plasma lipid and lipoprotein levels, glucose, hypertension, family history of CHD, and previous myocardial infarction. The plasma glucose levels of the CHD women were slightly higher than those of the CHD men, but the difference was not significant.
The E3/3 genotype occurred in 78.5% of the patients, followed by E3/4, E2/3, E2/4, and E4/4 genotypes that were observed in 9.5%, 9.5%, 1.9%, and 0.6% of the patients, respectively. Table 2 shows the distribution of apo E genotypes in men and women. No significant differences were observed between the genders in regard to the apo E genotype distribution. Table 3 lists the apo E allele distribution in men and women. No significant differences were observed between the genders.

The men with E3/3 genotype were more prone to have a family history of CHD compared to women with the same genotype (92% vs 71%, p = 0.035). No significant differences were noted between the apo E genotype distribution and the plasma lipid and lipoprotein concentrations or the history of myocardial infarction.

Discussion

In the present study of Greek Caucasian patients with CHD, no significant differences were observed in apo E genotype or allele distribution between matched men and women. However, in men with the E3/3 genotype, a family history of CHD was more frequent than in the corresponding women.

During the past decade, many papers suggested that there are ethnic differences in the frequencies of the apo E polymorphism [22]. Data from Asian and Caucasian populations presented a wide range of E2 allele frequency [18,23-26]. For example, in healthy subjects from Taiwan, the frequency of the E2 allele was 1.7% while in Southern European populations the E2 allele was found to be 7.3% in Italians and 7.9% in French [27,28]. The estimated E2 allele frequency in the healthy Greek population (8.1%) is close to the average Caucasian value (8.0%) [18,29]. In contrast, the E4 allele in healthy Greeks (10.2 %) is less prevalent than in most Northern European populations, such as 24.4% in Finns and 20.3% in Swedes, in whom the E4 allele may, in part, account for higher CHD mortality rates [20,29,30]. Dalloneville et al [26] reported that the frequency of the E4 allele is increased in populations at high risk of CHD and lower in geographic regions where the disease is less common. Even more conflicting data for apo E allele frequency have been found in specific populations (eg, patients with CHD) [4,10,32].
The possible influence of apo E polymorphism on the prevalence of CHD according to gender has not been investigated thoroughly. Keavney et al [33] evaluated the association of apo E polymorphism genotype with the myocardial infarction risk ratio in unmatched men and women and found no heterogeneity. Gender-related interactions are difficult to determine because of variations of hormonal status in women. There are changes in plasma lipid profile during pre- and post-menopause and during hormone replacement therapy [34]. At all ages, the prevalence of CHD is less in women than men. Gender-associated differences in plasma lipoprotein-lipid levels and in the prevalence of type II diabetes are partially responsible for the higher CHD in men. Indeed, men are characterised by less favorable plasma lipid profile (higher triglyceride and lower high-density lipoprotein cholesterol levels) and glucose-insulin homeostasis [35-37].

In the present study, the female CHD patients were all in postmenopausal status without hormonal replacement therapy. Furthermore, the CHD men and women were matched for age, body mass index, occurrence of hypertension, family history of CHD, and serum lipid-lipoprotein profile, in order to avoid confounding influences on CHD risk. Additionally, in both sexes the plasma lipid-lipoprotein levels were not influenced by hypolipidemic drug treatment. In the matched groups of male and female patients, no variations among different apo E genotypes were observed in relation to plasma lipid profiles or history of myocardial infarction.

In contrast to our findings, the E4 allele has been found to be associated with CHD in high-risk women [38]. On the other hand, van Bockxmeer and Mamotte [39] reported that Australian men (40 yr of age) who were homozygous for the E4 allele had a 16-fold increase in prevalence of CHD, compared to controls. Similarly, Scuteri et al [9] suggested that the presence of the E4 allele is a strong predictor of coronary events in men, but not in women. In a meta-analysis of 9 clinical studies that included 1971 men and 181 women, the risk of CHD for men with the E4 allele was estimated to be about 40% greater than in men without the E4 allele [4]. In our previous study, where 267 subjects with CHD were evaluated, the E4 allele frequency was similar in patients and 240 healthy controls [7]. It seems that, in the Greek population, the E4 allele does not influence CHD risk. As mentioned above, Greece belongs to the countries with low E4 allele frequency, which may explain the lack of association with CHD risk. In the present study, matched male and female patients with CHD had similar plasma lipoprotein and lipid levels and similar frequencies of the E4 allele. Thus, the putative effect of the E4 allele on CHD risk may not pertain to this study population.

Concerning the apo E genotype distribution in CHD subjects, Frikke-Schmidt et al [10] observed a higher frequency of the E3/4 genotype in CHD men compared to the general population. Frikke-Schmidt et al [10] found that E2/3 genotype was less frequent only in women with CHD, while the difference between being an E2/3 woman and an E3/4 or E4/4 woman amounted to a 4-fold risk.

In our study, no differences of the apo E genotype distribution were observed between the two genders. The comparison that we carried out was between CHD men and CHD women and not with the general population, since our aim was to evaluate gender differences in groups with otherwise similar clinical characteristics. However, the men of our population with the E3/3 genotype had a significant higher frequency of family history of CHD.

A limitation of this study is the small sample size, as many individuals were excluded from the study in order to match the CHD women and CHD men according to age, body mass index, and lipid profile. The sample size is statistically adequate to evaluate the differences of the genotype prevalence between the 2 genders. The 2 groups were not subdivided to investigate other interactions.

The results of this project support the hypothesis that there is no gender-specific influence of apo E polymorphism on CHD risk stratification in the Greek caucasian population. The sample size was adequate to evaluate differences of the genotype prevalence between genders and the subjects were matched to exclude possible confounding influences of other factors on CHD risk. The influence of the interaction between apo E polymorphism and gender on CHD risk may be ethnic-related rather than universal, which may explain the discrepancies
among the various studies. Further investigations are required before firm conclusions are reached.

References


