

Original Research

Genetic Variant in the CYP17 Gene and Risk of Premature Coronary Artery Disease

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Introduction: Sex hormones are well known to increase the risk of coronary artery disease (CAD). The CYP17 gene encodes the enzyme cytochrome P450c17 α , which functions at key steps during the process of human sex steroid hormone synthesis. A T/C polymorphism in the 5' promoter region of the CYP17 gene influences its expression and the resulting serum levels of androgens and estrogens. The aim of this case-control study was to investigate the role of a T/C CYP17 polymorphism in premature CAD and the occurrence of myocardial infarction (MI) in the Caucasian Greek population.

Methods: Our study group consisted of 230 CAD patients, aged less than 58 years, while 200 healthy individuals served as controls. The genotyping of the T/C CYP17 polymorphism was carried out using the PCR-RFLP method.

Results: The frequencies of TT, TC, and CC genotypes were 0.38, 0.42, and 0.20, respectively, in the patient group, and 0.35, 0.44, and 0.21, respectively, in the control group. Allele frequencies for the patient group were 0.58 and 0.42 for T and C, respectively, and 0.57 and 0.43, respectively, for the control group. Statistical analysis revealed no significant differences between patients and controls in genotype frequencies ($p=0.8746$) or allele frequencies ($p=0.6783$).

Conclusions: These findings do not support the hypothesis that the genetic variation T/C of the promoter of the CYP17 gene is an important contributing factor in the aetiology of premature CAD or occurrence of MI in the Caucasian Greek population.

Cardiovascular diseases are considered to be a major cause of mortality all over the world.¹ Coronary artery disease (CAD) is a complex multifactorial cardiovascular disease resulting from both genetic predisposition and the influence of several environmental risk factors. There is evidence that sex hormones influence the risk of developing CAD, although the differences in the pattern of disease between the two sexes have not been fully explained.^{2,3} Several risk factors have been associated with CAD development, including hypertension, hyperlipidaemia, diabetes and smoking, while male sex is considered to be one of the most important risk factors.

Several genes are known to be involved in the metabolic pathways of sex hormones. We hypothesized that the cytochrome P450c17 α gene (CYP17) could be a candidate gene predisposing to CAD development, as it plays a crucial role in sex hormone biosynthesis. The CYP17 gene is located on chromosome 10 (at 10q24.3) and encodes the cytochrome 450c17 α enzyme,^{4,5} which functions at key steps during the human sex steroid hormone synthesis process. It catalyses the conversion of pregnenolone to dehydroepiandrosterone, a precursor of testosterone and estrogens.⁶ A single base pair polymorphism T→C in the promoter region of CYP17 has been observed.⁷ The

frequency of the C allele in various populations ranges from 0.37 to 0.46.^{8,9} Serum levels of androgens and oestrogens have been shown to be elevated in subjects who carry the C allele.¹⁰⁻¹² Thus, it has been suggested that the C allele may provide additional promoter activity with an increased rate of CYP17 mRNA transcription.⁷

The present case-control study was conducted to determine whether the T/C polymorphism of CYP17 promoter is associated with the risk of premature CAD or occurrence of myocardial infarction (MI) in the Caucasian Greek population.

Methods

Study population

Our study group consisted of 430 unrelated individuals; 230 CAD patients and 200 healthy controls. All individuals enrolled in the study were Greek-Caucasians and informed consent was obtained from all participants. The patient group consisted of subjects aged less than 58 years presenting symptomatic CAD, documented by coronary angiography at the Onassis Cardiac Surgery Centre of Athens. CAD was defined as >50% luminal narrowing of at least one main coronary vessel, or a history of acute MI. Within the CAD group, we examined the T/C CYP17 polymorphism in the subgroup of 90 CAD patients who had previously suffered an MI. The diagnosis of MI was made according to the criteria of the World Health Organisation.

The control group consisted of individuals visiting the hospital who were found to be free from CAD. They had normal electrocardiograms with no evidence of cardiovascular disease, such as a history of angina pectoris or MI. Subjects were defined as hypertensive if their blood pressure was >140/90 mmHg and diabetic when they had fasting glucose >126 mg/dL. Hypercholesterolaemia was defined as having total cholesterol >220mg/dL. Family history was considered positive for CAD if at least one first-degree relative was diagnosed with CAD or MI by the age of 65 years. Finally, subjects were defined as smokers if they were current or past smokers.

The study was carried out in accordance with the ethical standards of the responsible institutional committee for human experimentation and with the Helsinki Declaration.

DNA isolation and genotyping

Genomic DNA was extracted from peripheral blood

leukocytes by a standard salting out method¹³ or using a DNA isolation kit (Macherey-Nagel GmbH & Co. KG, Germany). The genotyping of the T/C CYP17 polymorphism was carried out using the PCR-RFLP method, as previously described,¹⁴ using the following primers: forward, 5'-TCCTGAGCCCA-GATACCAT-3' and reverse, 5'-CCGCCCAGAGA-AGTCCT-3'. The PCR procedure was as follows: an initial denaturation step at 94°C for 2 min, followed by an amplification step for 35 cycles at 94°C for 30 s, 57°C for 40 s and 72°C for 40 min, followed in turn by a final extension step at 72°C for 7 min. The PCR products were digested with restriction enzyme MspAII (Promega Corp., USA) and separated by 3% agarose gel electrophoresis. Fragments were visualized by ethidium bromide staining and ultraviolet transillumination. The T→C transition in the variant allele (C) creates a new recognition site for the restriction enzyme MspAII, which permits identification of the wild type T and the mutant C allele. The 626 bp PCR product in the presence of the substitution T→C at -34bp of the CYP17 gene is cleaved into two fragments of 305 and 272 bp (C allele). The TT, TC, CC genotypes resulted in 577; 577, 305, 272; and 305, 272 bp digestion products, respectively.

Statistical analysis

Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS for Windows v10). Data are presented as total number, percentage, or mean. The chi-square test was used to test for independent relationships between categorical variables, such as genotype distribution. Differences in allele frequencies were tested for statistical significance at the 95% confidence interval using Fisher's exact test. A p-value <0.05 was considered statistically significant. The odds ratio (OR) was used as a measure of the strength of the association between allele frequencies and CAD or MI.

Results

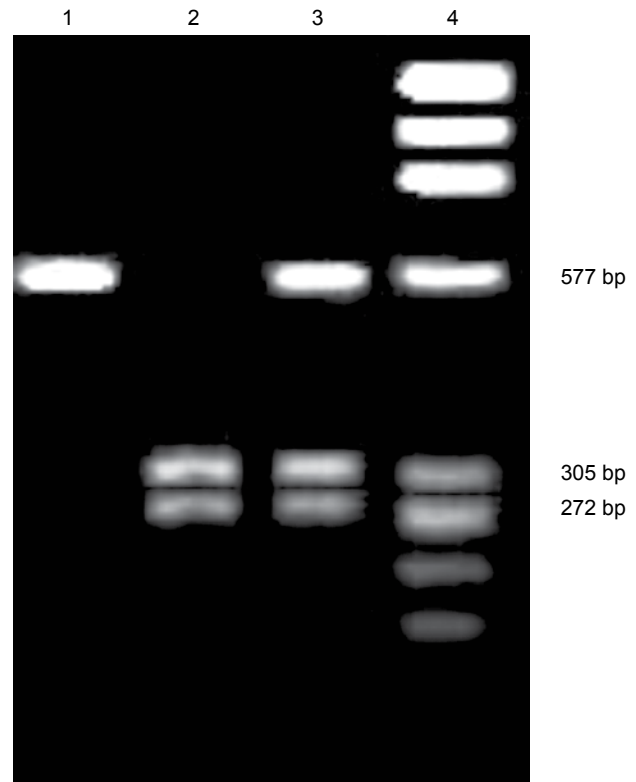
CAD

The clinical characteristics of the patients and the control groups are shown in Table 1. The mean age of the patient group was lower than that of the control group. An elderly healthy group would better represent a CAD-free population. The CAD patients had a significantly higher prevalence of risk

Table 1. Baseline characteristics of patients with coronary artery disease and control subjects.

	Patients n=230	Controls n=200	p
Mean age	53.2 (35-57)	68.6 (62-85)	
Male sex, n (%)	191 (83.0)	160 (80.0)	NS
Hypertension, n (%)	108 (46.9)	32 (16.0)	<0.0001
Diabetes, n (%)	57 (24.8)	9 (4.5)	<0.0001
Hypercholesterolaemia, n (%)	126 (54.8)	33 (16.5)	<0.0001
Smoking, n (%)	122 (53.0)	66 (33.0)	<0.0001
Family history, n (%)	74 (32.2)	23 (11.5)	<0.0001

factors such as hypertension, hyperlipidaemia, smoking, diabetes, and family history of CAD, compared with control subjects. All patients and controls were examined for the T/C CYP17 polymorphism. The three genotypes TT, TC, and CC are shown in Figure 1. The genotype distribution of the T/C CYP17 polymorphism among CAD patients and controls is shown in Table 2. The frequencies of TT, TC, and CC genotypes were 0.38, 0.42, and 0.20, respectively, in the CAD group and 0.35, 0.44, and 0.21, respectively, in the control group. The data of the two groups were statistically analysed using the chi-square test. The distribution of genotypes in each group was in Hardy–Weinberg equilibrium. Allele frequencies among patients and controls were 0.58 and 0.57, respectively, for the most frequent allele T, and 0.42 and 0.43, respectively, for the mutant allele C. These results show that the prevalence of the T/C CYP17 polymorphism was comparable between the patients and the control group. The statistical analysis showed that genotype or allele frequencies did not differ significantly between the patients and the control group ($p=0.8746$ for genotype frequencies comparison, $p=0.6783$ for allele frequencies comparison).

**Figure 1.** MspAII restriction pattern of the T/C CYP17 polymorphism. Lane 1: TT, lane 2: CC, lane 3: TC, lane 4: Ladder Φ X174DNA (HaeIII)**Table 2.** Genotype distribution and allele frequencies of the T/C CYP17 polymorphism in patients with coronary artery disease and control subjects.

Genotype	Patients n=230	Controls n=200	OR [95%CI]	p
TT	86 (0.38)	70 (0.35)		0.8746*
TC	97 (0.42)	88 (0.44)		
CC	47 (0.20)	42 (0.21)		
Allele:				
T	0.58	0.57	1.062 [0.8100-1.394]	0.6783†
C	0.42	0.43		

Data are expressed as number (percentage) of each genotype and gene frequency. *Probability value from chi-square test for homogeneity. †Probability value from Fisher's exact test for homogeneity.

MI

Within the CAD group, we examined the T/C CYP17 polymorphism in the subgroup of 90 CAD patients who had previously suffered an MI. The genotype distribution of this polymorphism among MI patients is shown in Table 3. The frequencies of TT, TC, and CC genotypes in the MI patient group were 0.29, 0.48, and 0.23, respectively. Allele frequencies in the MI group were 0.53 and 0.47, respectively, for the T and C alleles. Comparisons of the genotype and allele distribution between the subgroup of MI and the control group indicated that there were no significant differences between the two groups ($p=0.5910$ for genotype comparison, $p=0.3668$ for allele comparison). Similar results were observed for comparisons between the subgroup with MI and the remaining CAD patients, as seen in Table 4. Statistically significant differences were not found between the two subgroups ($p=0.1019$ for genotype frequencies, $p=0.0526$ for allele frequencies).

Discussion

Gender differences are known to affect the risk of developing cardiovascular disease.^{2,3} Despite the variations in total CAD mortality between different countries, the male-to-female ratio is constant at around two.¹⁵ The protective value of sex hormones appears to be sex-specific. In fact, oestrogens seem to be protective in women but detrimental in men. In women, early menopause is associated with an increased risk of CAD¹⁶ and MI is more common following bilateral oophorectomy,¹⁷ while high levels of endogenous oestrogen explain the low prevalence of CAD in premenopausal women.¹⁸ On the other hand, high levels of oestrogen and oestrone in men are associated with an increased risk of MI and CAD,¹⁹ while low levels of testosterone are also associated with an increased risk of CAD.^{20,21} Thus, the different balance of androgens and oestrogens in the two sexes is critical in determining the sex difference in mortality from CAD.

The central role of cytochrome P450 (CYP), a superfamily of enzymes, has been established regard-

Table 3. Genotype distribution and allele frequencies of the T/C CYP17 polymorphism in the subgroup of patients with myocardial infarction.

Genotype	Patients n=90	Controls n=200	OR [95%CI]	p
TT	26 (0.29)	70 (0.35)		0.5910*
TC	43 (0.48)	88 (0.44)		
CC	21 (0.23)	42 (0.21)		
Allele:				
T	0.53	0.57	0.8431 [0.5921-1.201]	0.3668†
C	0.47	0.43		

Data are expressed as number (percentages) of each genotype and gene frequency. *Probability value from chi-square test for homogeneity. †Probability value from Fisher's exact test for homogeneity.

Table 4. Genotype distribution and allele frequencies of the T/C CYP17 polymorphism in patients with myocardial infarction (MI) and patients with coronary artery disease (CAD) without MI.

Genotype	MI n=90	CAD without MI n=140	OR [95%CI]	p
TT	26 (0.29)	60 (0.43)		0.1019*
TC	43 (0.48)	54 (0.38)		
CC	21 (0.23)	26 (0.19)		
Allele:				
T	0.53	0.62	0.6809 [0.4659-0.9951]	0.0526†
C	0.47	0.38		

Data are expressed as number (percentages) of each genotype and gene frequency. *Probability value from chi-square test for homogeneity. †Probability value from Fisher's exact test for homogeneity.

ing the onset, progression and prognosis of CAD.²² The present case-control study was undertaken to investigate the influence of the T/C polymorphism of CYP17 gene on individual susceptibility to CAD or MI. This single-nucleotide polymorphism has been extensively studied in several hormone-related cancers (prostate, breast, ovarian, and endometrial cancer)²³⁻²⁶ and other hormone-dependent diseases, such as endometriosis,²⁷ with conflicting results. However two meta-analysis studies suggest that the T/C CYP17 polymorphism is associated with prostate cancer risk²⁸ but not with increased breast cancer risk.²⁹ Conversely, little is known concerning this CYP17 polymorphism in the field of cardiovascular diseases.

Our results in the Greek Caucasian population indicated no significant association between the T/C CYP17 polymorphism and susceptibility to CAD or MI. Genotype and allele frequencies did not differ significantly between the control group and the CAD or MI group. To our knowledge, this polymorphism has been studied for association with CAD only in the Slovenian population, also with negative results.³⁰ These findings indicate that the T/C polymorphism of CYP17 cannot be used as a genetic marker for premature CAD in our Greek Caucasian population. However, it is well known that the gene background differs among populations, and ethnic differences in genetic association studies are well documented.³¹⁻³⁴ Thus, more studies need to be performed in different populations in order to validate these results.

Conclusions

In the Greek Caucasian population the T/C CYP17 gene polymorphism does not contribute to the genetic susceptibility to premature CAD or the occurrence of MI. Therefore, this polymorphism is unlikely to be clinically useful for CAD risk assessment.

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