SHORT COMMUNICATION

Relationship between rs4674344 *CYP27A1* gene polymorphism and coronary artery disease in a Polish population

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Introduction Coronary artery disease (CAD) is a multifactorial condition. The efficacy of treatment and prevention methods depends on a better understanding of the genetic background of CAD as well as patient awareness about control of cardiovascular risk factors.[1]

The biosynthetic pathway of bile acids is the main mechanism of cholesterol catabolism in the human body.[2] CYP27A1, encoded by the *CYP27A1* gene (2q35), is a mitochondrial enzyme that is expressed in numerous tissues.[3] In the liver, CYP27A1 catalyzes the oxidation of cholesterol to 27-hydroxycholesterol (27-HC), which is an intermediate metabolite of bile acid synthesis.

The role of CYP27A1 in the context of cardiovascular diseases remains debatable. Some studies have demonstrated that CYP27A1 is involved in the mechanism protecting against the accumulation of cholesterol in macrophages.[4] On the other hand, 27-HC may enhance atherosclerotic plaque formation.[5] There are limited data on the participation of the *CYP27A1* variants in predisposing to cardiovascular diseases. So far, it has been demonstrated that the g.218805152 A>T polymorphism of the *CYP27A1* gene (rs4674344) was associated with an increased risk of metabolic syndrome and a decreased level of high-density lipoprotein (HDL) cholesterol.[6]

Because the role of the *CYP27A1* polymorphism in the context of CAD risk remains unclear, we decided to assess the potential association of the *CYP27A1* rs4674344 haplotype-tagging polymorphism with premature CAD and traditional risk factors for CAD.

Methods **Patients** We studied 445 white patients divided into 2 groups. The first group included 220 patients (59 women and 161 men) with angiographically confirmed premature CAD. The second group comprised 225 blood donors (62 women and 163 men) who served as controls with a negative family history of CAD defined as the absence of CAD, myocardial infarction, or stroke in at least one of the parents. Patients with CAD were recruited from the 1st Department of Cardiology at the Upper Silesian Center of Cardiology in Katowice and the 1st Department of Cardiac Surgery at the Upper Silesian Center of Cardiology in Katowice by the same clinician. Control individuals were selected among blood donors of the regional centers of blood donation and blood treatment (in Polish, Regionalne Centrum Krwiodawstwa i Krwiolecznictwa) in Katowice and Racibórz. The control group included only individuals without hypertension with a systolic blood pressure of less than 140 mm Hg and a diastolic blood pressure of less than 90 mm Hg on the day of blood collection. The levels of blood pressure higher than specified above were defined as hypertension.[7] Other inclusion and exclusion criteria were described in detail previously.[8] Patients and controls were recruited between 2001 and 2006.

The study was approved by the ethics committee of the Medical University of Silesia in

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Received: October 29, 2019.
Revision accepted: November 26, 2019.
Published online: November 27, 2019.
Kardiol Pol. 2020; 78 (1): 65-67
doi:10.33963/KP.15071

Katowice (Poland), and informed written consent was obtained from each participant.

Serum lipid measurement Lipid parameters such as total cholesterol, triglycerides, and HDL cholesterol levels were measured by enzymatic colorimetric methods (Analco, Warsaw, Poland). Low-density lipoprotein (LDL) cholesterol levels were calculated using the Friedewald formula.[9]

DNA extraction and genotyping Genomic DNA was extracted from peripheral leukocytes using the MasterPure genomic DNA purification kit (Epicenter Technologies, Madison, Wisconsin, United States). The *CYP27A1* polymorphism rs4674344 (g.218 805 152 A>T) was genotyped using the TaqMan Predesigned SNP Genotyping Assay kit (Thermo Fisher Scientific, Foster City, California, United States). Genotyping was performed using the 7300 Real-Time PCR System (Applied Biosystems, Foster City, California, United States).

Statistical analysis Statistical analysis was performed using the Statistica 13.0 (StatSoft, Tulsa, Oklahoma, United States) software. The Shapiro–Wilk test was used to check the normality of distribution of quantitative data. Comparison was performed by the Mann–Whitney test (nonnormal distribution) or the *t* test (normal distribution). Allele frequencies were deduced from the genotype distributions. Hardy–Weinberg equilibrium testing as well as comparisons of genotypes and allele frequencies between cases and control individuals were calculated using the χ^2 test. Statistical significance was accepted at a *P* value of less than 0.05. Odds ratios (ORs) with 95% CIs were computed using univariate and multiple logistic regression analysis after adjustment for traditional risk factors of CAD (male sex, cigarette smoking, hypertension, lipid abnormalities, diabetes mellitus, and overweight or obesity). If the number of individuals in the analyzed subgroups was zero, risk ratio values (95% CI) were used.

Results

Characteristics of the study groups The comparison of cases and controls showed that patients with CAD had increased total cholesterol, LDL cholesterol, and triglyceride concentrations as well as higher body mass index values. Furthermore, the serum concentration of HDL cholesterol was significantly lower in CAD patients than in controls.

Association between the *CYP27A1* polymorphism and coronary artery disease All genotype frequencies were compatible with the Hardy–Weinberg equilibrium (CAD group, $P = 0.999$; controls, $P = 0.66$). The analysis did not confirm the hypothesis about the association between genotypes and alleles of the rs4674344 polymorphism and CAD.

Association between the *CYP27A1* polymorphism and traditional risk factors for coronary artery disease There were no significant interactions between the genotypic variants of the rs4674344 polymorphism and traditional risk factors for CAD.

Association between the rs4674344 *CYP27A1* genotypes and hypertension The univariate analysis of the CAD group revealed an association between T-allele carrier status and hypertension in women ($\chi^2 = 5.81$; OR, 4.37; 95% CI, 1.26–15.19; $P = 0.02$; TABLE 1).

TABLE 1 Genotype distribution of the rs4674344 polymorphism in patients with and without hypertension

Genotype variant	Hypertension	No hypertension	χ^2	*P* value
Both sexes				
Number	125	95	–	–
AT + TT	99 (79.2)	65 (68.42)	3.3	0.07
AA	26 (20.8)	30 (31.58)	–	–
Women				
Number	43	16	–	–
AT + TT	35 (81.4)	8 (50)	5.81	0.02
AA	8 (18.6)	8 (50)	–	
Men				
Number	82	79	–	–
AT + TT	64 (78.05)	57 (72.15)	0.75	0.39
AA	18 (21.95)	22 (27.85)	–	–

Data are presented as number (percentage) unless otherwise indicated.

Discussion In our current study, we did not find an association between the rs4674344 polymorphic variant of the *CYP27A1* gene and the risk of CAD in the Polish population. The analysis showed an association of the T-allele carrier status with hypertension in the subgroup of women with CAD. There are no data on the effect of the rs4674344 polymorphism on the *CYP27A1* gene expression. However, available reports indicate that an elevated *CYP27A1* expression may increase the synthesis of 27-HC. In an animal model, this metabolite induced the expression of angiotensin I–converting enzyme and angiotensinogen.[10] Earlier reports also indicated a positive association between the expression of *CYP27A1* and the synthesis of marinobufagenin, an endogenous cardiotonic steroid acting as an endogenous inhibitor of Na^+/K^+–adenosine triphosphatase.[11] The decrease in the activity of Na^+/K^+–adenosine triphosphatase leads to an increase in the intracellular calcium concentration and, as a consequence, an increase in myocardial contractility and arterial contraction. The described pathomechanism can lead to hypertension.[12] Another study found that 27-HC acted antagonistically on the estrogen receptor, inhibiting estrogen-dependent synthesis of nitric oxide in arterial endothelial cells. This leads to a reduction of smooth muscle cell relaxation in the arterial wall, thus increasing the risk of hypertension and cardiovascular diseases.[13] The effect of 27-HC on estrogen receptors may also explain why the association of the *CYP27A1* gene polymorphism with hypertension was observed only in the female subgroup. Umetani et al[13] suggested that under conditions in which the 27-HC level is elevated relative to the estrogen level (eg, postmenopausal period or hypercholesterolemia), the protective effect of estrogens is reduced. Most of the women included in the current study were in the perimenopausal age and suffered from hypercholesterolemia, which may have increased the risk of hypertension.

To our knowledge, the association of the rs4674344 polymorphism with hypertension has previously been studied only in a Taiwanese population.[6] The obtained results were in line with our findings; however, the former study was performed solely in men.

In conclusion, the rs4674344 polymorphism of the *CYP27A1* gene potentially modifies the susceptibility to hypertension in Polish women with CAD.

ARTICLE INFORMATION

ACKNOWLEDGMENTS This work was supported by grants from the Medical University of Silesia (KNW-1-009/K/7/0, KNW-2-020/D/8/K).

CONFLICT OF INTEREST None declared.

HOW TO CITE Iwanicka J, Iwanicki T, Balcerzyk A, et al. Relationship between rs4674344 *CYP27A1* gene polymorphism and coronary artery disease in a Polish population. Kardiol Pol. 2020; 78: 65-67. doi:10.33963/KP.15071

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