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Association between rs5370 and rs9349379 polymorphisms and coronary artery disease in Polish population*

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ABSTRACT

Introduction: The 6p24 region modulates the risk of coronary artery disease (CAD), migraine headache, carotid artery dissection, fibromuscular dysplasia, and hypertension. It includes the *PHACTR1* and *EDN1* genes that are considered genetic *loci* for cardiovascular disease risk. The present study aimed to verify the hypothesis that polymorphisms of the 6p24 region (rs5370, rs9349379) shape the risk of CAD in the Polish population.

Materials and methods: The study included 490 Caucasian subjects divided into 2 groups. The 1st group consisted of 244 patients with angiographically confirmed premature CAD as CAD group and the 2nd group included 242 blood donors as controls. We performed serum lipid measurements and genetic analysis as well as statistical analysis containing biological interactions between genetic and environmental factors.

Results: The analysis showed an association between carrying the G allele of the rs9349379 polymorphism and a higher risk of myocardial infarction (MI) in <50-years-old CAD patients (OR: 2.16; 95% CI: 1.03–4.53; p = 0.040). Furthermore, carrying the T allele of rs5370 decreased the risk of hypertension in non-smokers (OR: 0.21; 95% CI: 0.05–0.82; p = 0.046). Moreover, our analysis showed that cigarette smokers carrying the T allele of the rs5370 polymorphism had more than a 9-times greater risk of MI (SI = 9.86, 95% CI: 2.17–35.12, p = 0.003) compared to allele GG homozygotes.

Conclusions: In conclusion, our results suggest that the genotypic variants of the rs9349379 *PHACTR1* predispose to MI in the subgroup under 50-years-old CAD group participants, and the variants of rs5370 *EDN1* modulate the risk of MI and hypertension depending on nicotinism.

Keywords: *EDN1*; *PHACTR1*; single nucleotide polymorphism; coronary artery disease.

INTRODUCTION

Coronary artery disease (CAD) remains a major cause of morbidity and death in developed countries [1]. Its multifactorial nature prompts the search for new genetic factors that predispose to the disease.

Genome-wide association studies indicate a potential role for the 6p24 region in shaping the risk of CAD, migraine headache, carotid artery dissection, fibromuscular dysplasia, and hypertension [2]. The region includes the *PHACTR1* and *EDN1* genes that are considered genetic *loci* for cardiovascular disease risk [3]. The *EDN1* gene (6p24.1) encodes a 21-amino acid peptide, endothelin-1 (ET-1) [4]. This protein is expressed in various tissues, regulating vascular tone, cell proliferation, and hormone secretion [5, 6]. Endothelin-1 1 released by endothelial and smooth muscle cells has a strong vasoconstrictor effect [7]. Increased ET-1 activity can promote vascular dysfunction through direct hemodynamic effects, vascular oxidative stress, inflammatory process, mitogenic stimulation of vascular smooth muscle cells, and fibrotic processes [8]. Endothelin-1 stimulates the production of reactive oxygen species which leads to the release of pro-inflammatory cytokines, chemokines, and adhesion molecules that are associated with the vascular inflammatory response [9]. Previous studies looking for an association between the rs5370 polymorphic variant of *EDN1* and hypertension, systolic blood pressure, and lipid metabolism parameters have provided inconsistent results [10, 11, 12, 13, 14, 15]. Moreover, several studies have shown that this variant of the *EDN1* gene could play an important role in revealing the phenotype of hearing impairment [16], asthma [17], preeclampsia [18], primary nephrotic syndrome [19], and vasovagal syncope [20].

Another variant selected for our study was rs9349379 of the *PHACTR1* gene, encoding phosphatase and actin regulatory protein 1 (PHACTR1). Literature data suggest an effector role for the *PHACTR1* gene relative to the process of angiogenesis [21], revealing the phenotype of atherosclerotic lesions, synthesis of extracellular matrix proteins [22], and inflammation-like conditions [23]. Previous studies also show the important role of this polymorphism in the pathogenesis of migraine [24], premature CAD [25], and coronary artery calcification [26]. In addition, the rs9349379 *locus* likely serves as an enhancer of the gene encoding ET-1 [3].

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Because the association of the selected polymorphisms in the context of CAD and cardiovascular events risk remains unclear, we decided to analyze these variants in the Polish population. Moreover, selected single nucleotide polymorphism (SNP) may modulate the expression of the *EDN1* and *PHACTR1* genes. The present study aimed to verify the hypothesis that polymorphisms of the 6p24 region (rs5370, rs9349379) shape the risk of CAD and cardiovascular events, such as myocardial infarction (MI). Given the poorly understood role of selected SNPs, we investigated not only their potential association with the risk of CAD and the clinical phenotype of CAD, but also their interactions with traditional CAD risk factors, such as being overweight, smoking, hypertension, and lipid disorders.

MATERIALS AND METHODS

This retrospective case-control study was conducted under STROBE guidelines. The case group consisted of patients with CAD and the control group included healthy blood donors. We have genotyped 2 SNPs.

Clinical material

The study included 490 Caucasian subjects divided into 2 groups. The 1st group consisted of 244 patients with angiographically confirmed premature CAD group, aged 44.40 ±5.97 years. The age range of probands was 25–55 years. The 2nd group included 242 blood donors as controls, aged 43.35 ±6.41 years, with a negative family history of CAD, MI, or stroke occurring in at least one of the parents. Coronary artery disease patients were recruited from the First Department and Clinic of Cardiology at the Upper Silesian Center of Cardiology in Katowice and the First Department of Cardiac Surgery at the Upper Silesian Center of Cardiology in Katowice by the same clinician. Controls were selected among blood donors of the Regional Centers of Blood Donation and Blood Treatment in Katowice and Racibórz.

According to the guidelines of the Regional Centers of Blood Donation and Blood Treatment, the control group included only subjects without hypertension with systolic blood pressure <140 mmHg and diastolic blood pressure <90 mmHg on the day of blood collection. Arterial hypertension was defined as systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg or if there was a significant history of hypertension. Other detailed inclusion and exclusion criteria were previously described [27].

The study groups were recruited between 2001–2011. This study was approved by the Ethics Committee of the Medical University of Silesia in Katowice (Poland) and informed written consent was obtained from each participant.

Serum Lipid Measurement

Lipid parameters like total cholesterol (TC), triglycerides (TG), and high density lipoprotein (HDL) levels were measured using the enzymatic colorimetric methods (Analco, Warsaw, Poland) and using a spectrophotometer (UV-VIS Mini 1240, Shimadzu). Low density lipoprotein (LDL) levels were calculated using the Friedewald formula [28] in subjects with TG levels below 4.4 mmol/L.

Genetic analysis

Genomic DNA was extracted from peripheral leukocytes using the MasterPure genomic DNA purification kit (Epicentre Technologies, Madison, WI, USA). The 6p24 region polymorphisms: intergenic variants rs5370 of the *EDTN1* gene and rs9349379 of the *PHACTR* gene were genotyped using the TaqMan®Predesigned SNP Genotyping Assay Kit (Applied Biosystems, Foster City, CA, USA). The 20-µL reaction mix consisted of 1 µL template DNA (15 ng/µL), 10 µL TaqMan®Genotyping Master Mix (Cat. #4371355), 1 µL probe (TaqMan® Pre-designed SNP Genotyping Assay), and 8 µL deionized water. The probe was diluted (1:1) in TE buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA) before the reaction. Polymerase chain reaction was performed according to the manufacturer's specifications. Genotyping was successful in 83–95% of participants, the accuracy of this analysis was checked by re-genotyping 15% of the samples, and the reproducibility of results was 100%.

Statistical analysis

For data analysis, the TIBCO® StatisticaTM 13.0 (STATSOFT, Poland) software was used. For quantitative data, the Shapiro–Wilk test was used to check the normality of distribution. Comparison of quantitative variables between the CAD group and controls was performed using the Mann–Whitney U test (for variables with non-normal distribution) or the Student's t-test (for variables with normal distribution). Allele frequencies were deduced from the genotype distributions. Hardy–Weinberg equilibrium testing and the comparisons of genotype and allele frequencies between the CAD group and controls were calculated using a χ^2 test. Statistical significance was accepted at $p < 0.05$. Odds ratios (ORs with 95% confidence intervals – CI) were computed using univariate and multiple logistic regression analysis after adjustment for age, sex, and traditional risk factors of CAD. If the number of individuals in the analyzed subgroups was 0, risk ratio values (95% CI) were used.

To determine possible biological interactions between the selected genotypes and traditional risk factors of CAD, the 4 x 2 table approach was used. Subjects without risk allele/genotype, not exposed to the specific traditional risk factor, were used as a reference group (00 code). They were compared with subgroups of subjects exposed to only one of the factors (01 – only traditional, 10 – only genetic) and with a subgroup exposed to both factors (11 code). The synergy measures in multiplicative and additive models were used to interpret the amount of interaction, according to the recommendations of Knol et al. [29] and Knol and VanderWeele [30]. Synergy indexes were calculated on the basis of OR values from 4 x 2 tables, using the following formulas:

— synergy index multiplicative:

$$SIM = OR11 \,/\, OR01 \;\; OR10$$

— Rothman's additive synergy index:

$$SI = OR11 - 1 / (OR01 - 1) + (OR10 - 1)$$

The relative excess risk due to interaction (RERI) parameter represents the relative risk increase resulting from interaction, calculated using the following formula:

$$RERI = OR11 - OR10 - OR01 + 1$$

The ranges of RERI vary from $-\infty$ to $+\infty$; RERI <0 means negative interaction, RERI = 0 means no interaction, and RERI >0 means positive interaction.

The proportion attributable to interaction (AP) of the combined effect due to interaction was calculated using the following formula:

$$AP = RERI / OR11$$

The ranges of AP are from –1 to +1. AP <0 means negative interaction, AP = 0 means no interaction, and AP >0 means positive interaction.

Asymmetric CIs for additive interaction parameters (SI) were determined using the model of Zou [31].

RESULTS

Groups characteristics

Clinical and biochemical characteristics of the CAD group and controls were performed. The CAD group had increased TC, LDL cholesterol, TG levels, as well as higher body mass index (BMI) and significantly lower HDL cholesterol levels than controls. Furthermore, in the CAD group, there were more nicotine smokers compared to the controls (Tab. 1).

Allele and genotype frequency in studied groups

The genotypes and allele frequencies of the rs5370 and rs9349379 polymorphisms are shown in Table 2. All genotype frequencies conformed to Hardy–Weinberg's equilibrium. The studied polymorphisms had a genotypic distribution consistent with Hardy–Weinberg's equilibrium.

Analysis of the association between the studied polymorphisms and clinical phenotypes of the coronary artery disease group

The analysis showed an association between carrying the G allele of the rs9349379 polymorphism and a higher risk of MI in the <50-years-old CAD subgroup (OR: 2.16; 95% CI: 1.03–4.53; p = 0.040; 155 CAD group participants – 112 with MI, 43 non-MI CAD group participants). Furthermore, carrying the T allele of the SNP rs5370 decreased the risk of hypertension in non-smokers (OR: 0.21; 95% CI: 0.05–0.82; p = 0.046 with Fischer exact; 53 non-smoking CAD group participants included – 33 with hypertension, 20 without hypertension).

There were no statistically significant associations between the genotypic variants of selected polymorphisms and severity of atherosclerosis (presence of multivessel coronary disease or critical occlusion >90%) observed during coronary angiography, left ventricular hypertrophy, and diabetes mellitus (data not shown).

Analysis of the association between the studied polymorphisms and lipid serum concentrations

There were no statistically significant associations between genotypic variants of selected polymorphisms and parameters of lipid metabolism in the controls (data not shown). This analysis was not performed on the CAD group due to lipid-lowering pharmacotherapy applied in all patients.

Interaction between the rs5370 polymorphism and cigarette smoking

The analysis showed a synergistic relationship between carrying the T allele of the rs5370 polymorphism and cigarette smoking on the risk of MI (Tab. 3). Cigarette smokers carrying the T allele of the rs5370 polymorphism had more than a 9-times greater risk of MI (SI = 9.86, 95% CI: 2.17–35.12, p = 0.003) compared to allele GG homozygotes. The relative increase of the risk resulting from the interaction of the above factors was positive (RERI = 0.76, 95% CI: –1.97–3.45). The AP value of this combined effect due to the interaction was 0.41, 95% CI: –1.51–1.08.

TABLE 1. Clinical and biochemical characteristics of coronary artery disease group participants and controls

Characteristics	Coronary artery disease group n = 244	Controls n = 242	Odds ratio* (95% CI)	p
Age (years), mean (SD)	44.40 ±5.97	43.35 ±6.41	–	0.065
Male gender – n (%)	162 (66.39%)	173 (71.49%)	–	0.589
Total cholesterol (mmol/L), mean (SD)	5.78 ±1.38	5.06 ±1.17	–	<0.000
High density lipoprotein (mmol/L), mean (SD)	1.12 ±0.38	1.45 ±0.56	–	<0.000
Low density lipoprotein (mmol/L), mean (SD)	3.89 ±1.20	2.96 ±1.20	–	<0.000
Triglicerydes (mmol/L), mean (SD)	1.83 ±0.98	1.38 ±0.71	–	<0.000
Body mass index (kg/m²), mean (SD)	27.11 ±4.30	26.08 ±3.72	–	0.015
Smoking – n (%)	138 (56.56%)	66 (27.27%)	3.97 (2.70–5.86)	<0.000
Myocardial infarction – n (%)	167 (68.44%)	–		

* univariate analysis; CI – confidence interval

TABLE 2. Analysis of genotype and allele frequencies of selected polymorphisms in coronary artery disease group participants and controls

Genotype/Allele	Coronary artery disease group n = 203 (%)	Controls n = 232 (%)	Inheritance model	Odds ratio (95% CI)	p
rs5370					
GG	136 (67.00%)	159 (68.53%)	dominant vs. GT + TT	0.93 (0.62–1.39)	0.732
GT	65 (32.02%)	64 (27.59%)	additive vs. GG	1.19 (0.78–1.80)	0.416
TT	2 (0.98%)	9 (3.88%)	additive vs. GG	0.26 (0.05–1.22)	0.068
GT + GG	201 (99.15%)	223 (96.12%)	recessive vs. TT	4.06 (0.87–19.00)	0.055
G	337 (83.00%)	382 (82.33%)	–	1.05 (0.74–1.49)	0.792
T	69 (17.00%)	82 (17.67%)	–	0.95 (0.67–1.36)	0.792
rs9349379	**CAD group n = 214 (%)**	**Controls n = 230 (%)**			
AA	62 (28.97%)	79 (34.35%)	dominant vs. AG + GG	0.78 (0.52–1.16)	0.224
AG	107 (50.00%)	107 (46.52%)	additive vs. AA	1.27 (0.83–1.95)	0.267
GG	45 (21.03%)	44 (19.13%)	additive vs. AA	1.30 (0.76–2.22)	0.329
AG + AA	169 (78.97%)	186 (80.87%)	recessive vs. GG	1.16 (0.78–1.71)	0.464
A	231 (53.97%)	265 (57.61%)	–	0.86 (0.66–1.12)	0.277
G	197 (46.03%)	195 (42.39%)	–	1.16 (0.898–1.51)	0.277

CI – confidence interval

TABLE 3. Interaction between carrying the G allele of the rs5370 polymorphism and cigarette smoking

T allele, rs5370	Cigarette smoking	CAD group group MI	CAD group Non-MI	Odds ratio	95% confidence intervals	
					lower limit	upper limit
0	0	39	16	1		
0	1	51	19	1.10	0.50	2.41
1	0	12	5	0.98	0.30	3.25
1	1	36	8	1.85	0.71	4.83

CAD group MI – myocardial infarction in the coronary artery disease group; CAD group Non-MI – coronary artery disease without myocardial infarction in the coronary artery disease group

DISCUSSION

Our results show the importance of carrying the G allele of the rs9349379 *PHACTR1* variant in shaping the risk of MI in the under-50-year-old subgroup. Previous research on the Chinese population has shown an association between the GG genotype of the rs9349379 and CAD risk [32]. Moreover, this functional polymorphism determines increased gene expression, which promotes coronary vasoconstriction and plays an important role in the pathogenesis of coronary microvascular dysfunction. Carrying the G allele of rs9349379 has been associated with impaired myocardial perfusion and increased ET-1 levels [33], independently increasing the risk of adverse cardiovascular events [34]. Endothelin-1 activates the ETA receptor, which promotes the progression of inflammation and vascular endothelial dysfunction [35].

Our analysis also revealed an interesting relationship relative to rs5370 *EDN1*. Cigarette-smoking carriers of the T allele of the rs5370 *EDN1* polymorphism were significantly predisposed to MI. In the group of non-smoking patients, the T allele showed a protective effect against high blood pressure. Previous studies indicate the presence of higher plasma ET-1 concentrations in carriers of the T rs5370 allele relative to GG homozygotes. The importance of ET-1 concerning blood pressure is dependent on the distribution and mutual contribution of ETA and ETB receptors. Mediated by them, ET-1 can act as a vasoconstrictor for specific blood vessels or a systemic vasodilator, thereby reducing the risk of hypertension.

Also noteworthy are the possible interactions between the studied polymorphic variants. Epigenomic profiling revealed an enhancer signature of the rs9349379 *PHACTR1* variant within the aorta, suggesting a regulatory function for this SNP in the vasculature. The reported variant regulates the expression of the *EDN1* gene, located 600 kb upstream of *PHACTR1* [2]. However, analyzing the associations shown in this study, we are inclined to believe that the major factor shaping the risk of acute

cardiovascular incidents and hypertension in our group was cigarette smoking. In our previous work, we highlighted the role of nicotinism in the pathomechanism of atherosclerotic CAD [36]. Cigarette smoke contains many oxidizing agents and is an important source of free radicals, which contribute to both the development of atherosclerosis and an increase in the rate of cardiovascular incidents among smokers [37].

To summarize our results, carrying the G allele of rs9349379 *PHACTR1* predisposed to MI in the subgroup of under 50-year-old CAD patients. Referring to the role of the rs5370 *EDN1* variant, presumably, the greatest significance in shaping the risk of infarction or hypertension in the studied group of patients was nicotinism and not the mentioned genetic factor.

The presented results should be approached with some caution due to the small group size and the adopted definition of nicotinism which does not consider detailed information on the daily dose and duration of exposure to cigarette smoke.

Further perspectives

We are going to continue the research to better understand the genetic basis of CAD and the impact of selected genetic variants in the context of survival analysis.

CONCLUSIONS

In conclusion, our results suggest that the genotypic variants of the rs9349379 *PHACTR1* predispose to MI in under 50-years-old CAD patients, and the variants of rs5370 *EDN1* modulate the risk of MI and hypertension depending on nicotinism.

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