ASSOCIATION OF THE *PDE4D* GENE VARIANT WITH SELECTED MARKERS IN INDIVIDUALS WITH ISCHAEMIC HEART DISEASE: A PILOT STUDY

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SUMMARY

Objective: The aim of the study was to evaluate the variant (rs2910829) of the *PDE4D* gene in relation to its influence on biochemical, anthropometric and physiological parameters in patients with coronary artery disease and healthy subjects of the Eastern Slovak population.

Methods: The male group consisted of 72 individuals and the female group consisted of 132 individuals. On the basis of clinical screening the subjects were divided into two groups – with ischaemic heart disease and control group. Genomic DNA was isolated from peripheral blood using a commercial NucleoSpin® Blood Machenery-Nagel kit. Molecular genetic analysis of the polymorphism under study was performed using the StepOne™ Real-Time PCR System instrument. The lipid profile markers TC, HDL, LDL, TG were measured by Cobas Integra 400 plus biochemical analyser, and systolic and diastolic blood pressure using a digital blood pressure monitor. Among anthropometric parameters, body height and weight, waist and hip circumference were measured and BMI and WHR indices were calculated.

Results: A statistically significant ($p = 0.018$) possible association between the mutant T allele and ischaemic heart disease was found in men. In women, we found a statistically significant difference in the systolic ($p = 0.013$) and diastolic blood parameters ($p = 0.005$) in the CC genotype. In the group of women, we found statistically significant differences in all observed anthropometric parameters and in LDL and TC markers. In the group of men divided on the basis of BMI, statistical significance was found in systolic blood pressure ($p = 0.028$). In the group of women with ischaemic heart disease, we found a negative correlation between BMI and HDL.

Conclusion: The study contributes to new findings of the representation of genotypes and alleles of the rs2910829 *PDE4D* gene polymorphism in the Slovak population. This is a pilot study. Interactions between genotype and observed anthropometric, physiological and biochemical markers were confirmed.

Key words: BMI, cardiovascular disease, lipids, PDE4D gene, systolic, diastolic

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INTRODUCTION

Ischaemic heart disease (IHD) is a complex multifactorial disease that is one of the most common causes of morbidity and mortality worldwide. It is a dynamic process of atherosclerosis of the coronary arteries or functional changes in the coronary circulation, defined by the presence of one or more obstructive plaques that reduce coronary blood flow, causing myocardial ischaemia and subsequent heart failure (1, 2). The incidence and mortality rates associated with IHD are declining in most economically developed and developing countries, but absolute numbers are steadily increasing (3). The disease has become a global public health problem (4). According to data from Eurostat (database), the average mortality rate from coronary heart disease in the European Union is 132/100 thousand inhabitants, with Slovakia achieving more than three times the European average of 433/100 thousand inhabitants. Countries with high mortality rates from this disease include Lithuania, Latvia, the Czech Republic, and Hungary. In contrast, in the United Kingdom, the Netherlands and Ireland, IHD mortality rates have declined by more than 60% (3). The incidence and prevalence of IHD varies based on age, sex, ethnicity, socioeconomic, demographic, environmental, and genetic factors and the relationships between them (5). Scientific studies have reported that smoking, alcohol consumption, sedentary lifestyle, high BMI, hyperlipidaemia, high blood pressure, and unbalanced diet contribute to the development of IHD and the development of cardiovascular diseases (6). The phosphodiesterase 4D (*PDE4D*) gene plays an important role in the degradation of cyclic adenosine monophosphate (cAMP) (7). The function of cAMP in atherosclerosis processes is currently

not fully understood. Scientific studies point to a key role of cAMP in the development of acute coronary syndromes, unstable angina, acute myocardial infarction and stroke, by modulating platelet aggregation, thrombus formation and expression of metalloproteinases (8). *PDE4D* gene expression is expressed in B and T lymphocytes in cells of several organs (brain, lung, kidney). The enzymatic activity of *PDE4D* may play an important role in stroke risk through its involvement in inflammation and the generation of a physiological response related to vascular injury and in angiogenesis (9, 10). The aim of this study was to evaluate the variant (rs2910829) of the *PDE4D* gene in relation to its impact on biochemical, anthropometric and physiological parameters in patients with coronary artery disease and healthy individuals of the Eastern Slovak population.

MATERIALS AND METHODS

Research File

The study population consisted of 204 subjects who were divided into two groups based on gender and medical examination: a control group (CG) (without diagnosed ischaemic heart disease) and a group of subjects diagnosed with ischaemic heart disease (IHDG). The group of subjects diagnosed with ischaemic heart disease consisted of 66 women aged 52–87 years and 36 men aged 47–85 years. The control group also consisted of 66 women aged 49–89 years and 36 men aged 42–83 years. Criteria for inclusion of subjects in the IHDG: patients diagnosed with coronary artery disease had selective coronary angiography with findings of coronary artery atherosclerosis or had documented resolution of acute coronary syndrome. These patients were using antihypertensives and hypolipidemic therapy (statins). Their blood pressure readings were measured, lipid profile analysis was performed, and a questionnaire was administered on the influential factors of cardiovascular disease (lifestyle of the individuals). Exclusion criteria: individuals with cancer, renal insufficiency and severe hepatic disease, pulmonary heart disease, hyperthyroidism, severe infection, hormone or other immunization within the past month, patients with inhibitors, active tuberculosis were excluded from the study. The scientific study was performed over a period of months (June–December 2022). All individuals in the study population voluntarily provided a peripheral blood sample and written informed consent. They were informed that their sample would be anonymous and used only for research purposes. This scientific study was conducted on the basis of the approval of the Ethics Committee of the University of Prešov No. 2/2013 and No: ECUPO42022PO-1/7. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Molecular Genetic Analysis

Blood for molecular genetic analysis was collected in SARSTEDT tubes (SARSTEDT AG & Co. KG, Germany) 2.7 ml, K_3EDTA. DNA was isolated from the blood using the NucleoSpin® Blood isolation kit (Machenery-Nagel, Germany). The DNA concentration was verified using a NanoDrop™ 2000 UV-Vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Genotyping analysis was performed with the StepOne™ Real-Time PCR System (Applied Biosystems, CA). Genotyping was performed using TaqMan genotyping SNP assay (C___2820061_10) (Thermo Fisher Scientific, Waltham, MA, USA) based on a standard protocol. The mutant genotype was verified by repeated measurements.

Biochemical Analysis

Biochemical analysis was performed in peripheral blood samples collected in SARSTEDT tubes (SARSTEDT AG & Co. KG, Germany) 5.5 ml. Subsequently, blood serum was separated by centrifugation – CENTRONIC BL-II centrifuge (JP Selecta, Spain) at a speed of 5,000 rpm for 10 minutes. Using a Cobas Integra 400 plus biochemical analyser (Roche, Switzerland), we measured the levels of selected lipid profile markers – total cholesterol (TC), high density lipoprotein (HDL), low-density lipoprotein (LDL), and triacylglycerols (TG) in the blood serum.

Anthropometric and Blood Pressure Measurements

The following anthropometric parameters were measured in the subjects: body weight – using a digital personal scale with an accuracy of 100 g (DM – 117 Dimarson, Hungary), body height – digital altimeter (Soehnle, USA), waist and hip circumference was measured by means of a textile waist tape measure. On the basis of the obtained data, we calculated body mass index (BMI): body weight (kg)/body height (m^2), waist circumference (cm)/hip circumference (cm) (WHR). Blood pressure was measured using a digital blood pressure monitor (Tensoval duo control, Hartmann, Germany) in all subjects involved in the study. The normal physiological blood pressure values were considered to be systolic blood pressure 120–129 mmHg and diastolic blood pressure 80–84 mmHg.

Statistical Analysis

The measured data were processed by Microsoft Excel 2016, then the data were statistically evaluated by Statistica ver. 12. We used the parametric Student's t-test to detect differences in individual parameters between groups. To detect the difference between the two sets, their medians, we used the non-parametric Mann-Whitney U-test method. We used a nonparametric analysis of variance, the Kruskal-Wallis test, to detect differences in means between a larger number of sets. We used Spearman's correlation coefficient to detect correlation relationships between two parameters. We used the chi-squared test (χ^2 test) to statistically evaluate the results of genotype and allele frequency representation between the control group and the group of subjects with coronary artery disease. The odds ratio (OR) and confidence interval (CI) were calculated using the online calculator MedCalc statistical software and Social Science Statistical. Statistical significance was set at $p < 0.05$.

RESULTS

A total of 204 individuals were included in the scientific study. The male group consisted of 72 individuals and the female group consisted of 132 individuals. Molecular genetic analysis

Table 1. *Distribution of genotypes and alleles in the studied groups of individuals based on sex*

	Men		Women	
	CGM n = 36 n (%)	**IHDM n = 36 n (%)**	**CGW n = 66 n (%)**	**IHDW n = 66 n (%)**
Genotypes				
CC	9 (25.00)	4 (11.11)	11 (16.67)	9 (13.64)
CT	19 (52.78)	15 (41.67)	34 (51.51)	36 (54.54)
TT	8 (22.22)	17 (47.22)	21 (31.82)	21 (31.82)
p-value for HWE	0.944	0.969	0.907	0.581
X^2	5.633		0.257	
p-value	0.060		0.879	
Alleles				
C	37 (51.39)	23 (31.94)	56 (42.42)	54 (40.91)
T	35 (48.61)	49 (68.06)	76 (57.58)	78 (59.09)
X^2	5.600		0.062	
OR (95% CI)	2.252 (1.144–4.434)		1.064 (0.652–1.736)	
p-value	0.018*		0.803	

CGM – control group of men; IHDM – group of men with ischaemic heart disease; CGW – control group of women; IHDW – group of women with ischaemic heart disease; HWE – Hardy-Weinberg equilibrium; statistical significance *$p < 0.05$; χ^2 – chi-square test; OR – odds ratio; CI – confidence interval

was performed to determine and evaluate the representation and frequency of alleles and genotypes of the rs2910829 *PDE4D* gene polymorphism in both study groups (Table 1). In the group of men with ischaemic heart disease, the TT genotype was represented in the highest frequency (47.22%), in the control group it was the CT genotype (52.78%). In the female group, the CT genotype was represented at the highest frequency in the control group (51.51%), and the group diagnosed with ischaemic heart disease (54.54%). We did not observe any deviation from Hardy-Weinberg equilibrium in either of our study groups. Through chi-square test, we found no statistical significance in the representation of genotypes in our study groups of individuals ($p = 0.060$, $p = 0.879$). In the female group, we found that the most frequent allele was the mutant allele T. It was frequent 57.58% in the control group and 59.09% in the group of individuals diagnosed with coronary artery disease. In the male group, the mutant T allele was present at the highest frequency in the group of individuals diagnosed with ischaemic heart disease (68.06%), but in the control group it was the ancestral C allele (51.39%). Statistical evaluation of the results by calculating the odds ratio revealed a statistically significant ($p = 0.018$) possible association between the mutant T allele and coronary artery disease in the male group. This was not statistically confirmed in women ($p = 0.803$). The results of anthropometric, physiological and biochemical parameters measured are shown in Table 2. Using Student's t-test, we found a statistically significant difference ($p = 0.041$) between the control group and the group with ischaemic heart disease in men only in the anthropometric parameter WHR. On the contrary, in the female group ($n = 132$), we found a statistically significant difference in all observed, measured and calculated anthropometric parameters except age. The mean systolic blood pressure values of men and women in the control group and in the group of individuals diagnosed with ischaemic heart disease were higher, than the normal physiological systolic blood pressure values of 120–129 mmHg. Using the Student's t-test, we found a statistically significant difference ($p = 0.009$) between the control group and the group with ischaemic heart disease in the diastolic blood pressure parameter in the women's group. The mean values of biochemical parameters TG, TC, LDL in male and female group were consistent with the reference values. HDL values in both groups of women and in men with ischaemic heart disease were below the lower limit of reference values (women = 1.6 mmol/L, men = 1.4 mmol/L). In the group of women, when we evaluated the variant (rs2910829) of the *PDE4D* gene in relations to biochemical markers, we found a statistically significant difference between CG and IHDG in the LDL parameter ($p = 0.012$) in the TT genotype, by the Mann-Whitney U-test. In CT genotype, a statistically significant difference was found in TC ($p = 0.018$) and LDL ($p = 0.045$) parameters (Table 3). In the male group, the Mann-Whitney U-test revealed a statistically significant difference between control group and group with ischaemic heart disease only in CT genotype, for the parameters TC ($p = 0.006$), LDL ($p < 0.001$) and TG ($p = 0.016$) (Table 4). When the effect of the variant (rs2910829) of the *PDE4D* gene on anthropometric parameters was investigated, again in the male group, in the CT genotype, we found a statistically significant difference in the parameters – waist circumference ($p = 0.023$) and hip circumference ($p = 0.042$). Through the Kruskal-Wallis non-parametric analysis of variance, in the group of men with coronary artery disease, we found statistically significant differences between TT and CT genotypes in the anthropometric parameters waist circumference ($p < 0.05$) and hip circumference ($p < 0.01$). In the group of women, we detected a statistically significant difference between control group and group with ischaemic heart disease in the anthropometric parameters – waist circumference and BMI and WHR indices in TT and CT genotypes by statistical analysis. A statistically significant difference (Mann-Whitney U-test), in terms of physiological parameters (systolic and diastolic

blood pressure) monitoring, was found in the group of women in genotype CC (Table 3). In our study, we detected a negative correlation between BMI and HDL, and a positive correlation between BMI and TG in a group of women with coronary artery disease. In the control group of women, we found positive correlation between BMI and TC, BMI and TG, waist circumference and TG, and negative correlation between waist circumference and HDL ($p < 0.05$).

DISCUSSION

Cardiovascular disease is currently the leading cause of morbidity and mortality worldwide (11, 12). The pathophysiology of cardiovascular disease is the result of a complex interaction between factors such as smoking, hypertension, hyperlipidaemia, diabetes mellitus, as well as genetic predisposition (13). The *PDE4D* gene is located on chromosome 5q12. Polymorphisms of this gene are mainly associated with ischaemic stroke and cardiovascular diseases in different populations with controversial findings (14–17). In a study by Sinha et al. (16), two polymorphisms (rs966221 and rs2910829) of the *PDE4D* gene were observed in an Indian population in patients with coronary artery disease and controls. They did not directly detect an association with the disease. They found an association of both polymorphisms with hypertriglyceridemia. The CC genotype was represented in the highest frequency in both study groups (cases 94.62%, controls 91.67%). In our study, the CC genotype presented with the lowest frequency in both female groups analysed (cases 13.64%, controls 16.67%) and also in the group of men diagnosed with coronary artery disease (11.11%). Another study (18) investigated *PDE4D* gene polymorphisms (SNP87, SNP56, SNP89, SNP83, SNP41) and their association with ischaemic stroke. The authors point out that only one of these studied polymorphisms (rs2910829) was significantly associated with cardioembolic stroke among whites and blacks in Kentucky and Ohio. Milton et al. (15) report that they did not find a significantly significant association of the rs2910829 *PDE4D* gene polymorphism with stroke. A study of the genotype and allele frequencies of the rs2910829 *PDE4D* gene polymorphism was conducted by Ma et al. (19) in the Uyghur and Han ethnic groups in China. The order of genotype frequencies of the rs2910829 *PDE4D* gene in the experimental and control groups was CC > CT > TT. There was no statistically significant difference in the frequency distribution of genotypes ($\chi^2 = 5.765$, $p = 0.056$; $p > 0.05$). The distribution of the C allele in the experimental group was significantly higher than that in the control group ($\chi^2 = 4.672$, $p = 0.031$; $p < 0.05$). Individuals with the C allele had a higher risk of cerebral infarction than those with the T allele, with an OR of 1.314 (95% CI: 1.025–1.685; $p < 0.05$). In our study, we found the following order of genotype frequencies in the group of women (control group and group with ischaemic heart disease), CT > TT > CC ($\chi^2 = 0.257$, $p = 0.879$; $p > 0.05$). In the control group of men, it was CT > CC > TT and in the group of men with ischaemic heart disease (TT > CT > CC) ($\chi^2 = 5.633$, $p = 0.060$; $p > 0.05$). In the group of women, we found that the most abundant allele was the mutant allele T. It occurred at a frequency of 57.58% in the control group and 59.09% in the group with ischaemic heart disease. In the male group, the mutant T allele was present at the highest frequency in the group with ischaemic heart disease (68.06%), but in the control group it was the ancestral C allele (51.39%). Statistical evaluation of the results by calculating the odds ratio revealed a statistically significant ($p = 0.018$) possible association between the mutant T allele and coronary heart disease in the male group. These results cannot be interpreted directly in relation to the size of the studied population. A study (19) found no statistically significant differ-

Table 2. *Characteristics of anthropological, biochemical and physiological parameters in patients and controls*

Variable	Women			Men		
	IHDW n = 66 Mean (SD)	CGW n = 66 Mean (SD)	p-value	IHDM n = 36 Mean (SD)	CGM n = 36 Mean (SD)	p-value
Body height (cm)	158.48 (7.39)	161.64 (5.80)	0.007**	172.31 (8.30)	174.53 (5.26)	0.179
Body weight (kg)	82.82 (15.81)	77.70 (12.51)	0.041*	89.92 (17.57)	91.31 (15.88)	0.726
Waist circumference (cm)	114.48 (13.27)	98.92 (11.47)	< 0.001***	115.36 (9.91)	110.19 (12.90)	0.060
Hip circumference (cm)	110.62 (12.00)	104.88 (10.36)	0.004**	107.69 (9.04)	106.19 (7.33)	0.442
BMI (kg/m^2)	32.95 (5.87)	26.71 (4.32)	< 0.001***	30.19 (5.04)	30.02 (5.36)	0.889
WHR	1.04 (0.09)	0.95 (0.08)	< 0.001***	1.07 (0.09)	1.04 (0.07)	0.041*
Age (years)	72.11 (7.75)	70.71 (7.86)	0.307	67.92 (10.25)	61.03 (7.65)	0.002**
Systolic blood pressure (mm/Hg)	133.44 (22.02)	131.12 (19.59)	0.523	133.11 (19.84)	134.66 (13.53)	0.700
Diastolic blood pressure (mm/Hg)	76.95 (10.41)	81.39 (8.90)	0.009**	77.64 (10.78)	81.47 (10.06)	0.123
TC (mmol/L)	3.60 (1.40)	4.13 (0.89)	0.010**	3.44 (1.43)	4.24 (1.17)	0.010**
HDL (mmol/L)	1.12 (0.23)	1.04 (0.38)	0.188	0.90 (0.30)	1.04 (0.27)	0.045*
LDL (mmol/L)	1.79 (0.86)	2.32 (0.79)	< 0.001***	1.70 (0.81)	2.59 (0.08)	0.001***
TG (mmol/L)	0.92 (0.56)	1.06 (0.54)	0.131	0.86 (0.41)	1.22 (0.73)	0.013*

CGM – control group of men; IHDM – group of men with ischaemic heart disease; CGW – control group of women; IHDW – group of women with ischaemic heart disease; BMI – body mass index; WHR – waist to hip ratio; TC – total cholesterol; HDL – high-density lipoprotein; LDL – low density lipoprotein; TG – triglycerides; statistical significance *p < 0.05, **p < 0.01, ***p < 0.001

Table 3. *Anthropometric characteristics and indexes, biochemical and physiological parameters of studied women according to genotypes of rs2910829 PDE4D gene polymorphism*

Parameters	CC			CT			TT		
	IHDW (n = 66) Mean (SD)	CGW (n = 66) Mean (SD)	p-value	IHDW (n = 66) Mean (SD)	CGW (n = 66) Mean (SD)	p-value	IHDW (n = 66) Mean (SD)	CGW (n = 66) Mean (SD)	p-value
Number	9	11	–	36	34	–	21	21	–
Body height (cm)	156.56 (4.48)	160.55 (6.13)	0.107	158.08 (7.65)	161.40 (5.98)	0.048*	160.00 (7.92)	162.60 (5.46)	0.193
Body weight (kg)	81.44 (17.95)	83.00 (13.56)	0.648	83.92 (17.19)	76.97 (11.58)	0.052	81.52 (12.71)	76.10 (13.29)	0.247
Waist circumference (cm)	113.22 (14.93)	106.45 (11.00)	0.382	113.83 (13.12)	97.88 (11.48)	< 0.001***	116.14 (13.32)	96.67 (10.55)	< 0.001***
Hip circumference (cm)	112.11 (14.01)	110.45 (10.74)	0.761	109.03 (12.42)	103.76 (10.07)	0.056	112.71 (10.46)	103.76 (10.16)	0.011*
BMI (kg/m^2)	33.06 (6.23)	32.15 (4.57)	0.713	33.55 (6.46)	29.54 (4.08)	0.002**	31.87 (4.65)	28.70 (4.29)	0.047*
WHR (cm)	1.01 (0.05)	0.97 (0.08)	0.068	1.05 (0.09)	0.94 (0.08)	< 0.001***	1.03 (0.11)	0.93 (0.09)	< 0.001***
Systolic blood pressure (mm/Hg)	120.11 (16.47)	144.64 (19.17)	0.013*	134.97 (23.08)	126.65 (20.40)	0.115	136.52 (21.00)	131.29 (15.54)	0.326
Diastolic blood pressure (mm/Hg)	70.89 (8.19)	82.64 (6.68)	0.005**	77.50 (10.37)	79.59 (9.02)	0.372	78.62 (10.83)	83.67 (9.43)	0.162
TC (mmol/L)	4.18 (1.27)	3.99 (0.57)	0.679	3.55 (1.45)	4.25 (0.89)	0.018*	3.42 (1.37)	4.01 (1.04)	0.222
HDL (mmol/L)	1.25 (0.36)	0.95 (0.46)	0.287	1.10 (0.27)	1.04 (0.41)	0.433	1.09 (0.20)	1.10 (0.30)	0.571
LDL (mmol/L)	1.88 (0.85)	2.21 (0.44)	0.254	1.80 (0.92)	2.23 (0.82)	0.045*	1.73 (0.79)	2.51 (0.86)	0.012
TG (mmol/L)	1.28 (0.85)	1.13 (0.62)	0.761	0.89 (0.55)	1.08 (0.57)	0.174	0.81 (0.36)	1.01 (0.45)	0.102

CGW – control group of women; IHDW – group of women with ischaemic heart disease; CC, CT, TT – genotypes; statistical significance *p < 0.05, **p < 0.01, ***p < 0.001

Table 4. *Anthropometric characteristics and indexes, biochemical and physiological parameters of studied men according to genotypes of rs2910829 PDE4D gene polymorphism*

Parameters	CC			CT			TT		
	IHDM (n = 36) Mean (SD)	CGM (n = 36) Mean (SD)	p-value	IHDM (n = 36) Mean (SD)	CGM (n = 36) Mean (SD)	p-value	IHDM (n = 36) Mean (SD)	CGM (n = 36) Mean (SD)	p-value
Number	4	9	–	15	19	–	17	8	–
Body height (cm)	169.75 (4.50)	174.11 (5.86)	0.213	174.60 (9.21)	174.32 (5.69)	0.715	170.88 (7.99)	175.50 (3.82)	0.046*
Body weight (kg)	83.25 (11.64)	90.11 (10.09)	0.485	95.13 (18.22)	92.95 (18.28)	0.544	86.88 (17.70)	88.75 (16.38)	0.815
Waist circumference (cm)	111.75 (8.66)	109.22 (9.11)	0.757	120.60 (9.57)	111.79 (13.56)	0.023*	111.59 (8.75)	107.50 (15.75)	0.414
Hip circumference (cm)	103.25 (3.77)	104.78 (4.15)	0.587	113.47 (8.66)	107.26 (8.87)	0.042*	103.65 (7.53)	105.25 (6.32)	0.769
BMI (kg/m^2)	28.80 (2.72)	29.74 (3.18)	0.699	31.20 (5.86)	30.66 (6.32)	0.665	29.63 (4.73)	28.81 (5.14)	0.541
WHR (cm)	1.08 (0.10)	1.04 (0.05)	0.699	1.07 (0.09)	1.04 (0.07)	0.358	1.08 (0.08)	1.02 (0.10)	0.137
Systolic blood pressure (mm/Hg)	141.25 (14.36)	141.67 (16.20)	0.815	128.00 (19.52)	131.30 (9.09)	0.611	135.71 (21.00)	134.75 (17.57)	0.912
Diastolic blood pressure (mm/Hg)	77.50 (9.57)	85.33 (8.79)	0.205	77.27 (10.87)	80.58 (10.95)	0.451	78.00 (11.55)	79.25 (9.08)	0.747
TC (mmol/L)	4.00 (1.53)	4.56 (0.60)	0.487	3.22 (1.42)	4.43 (1.23)	0.006**	3.50 (1.45)	3.44 (1.26)	0.977
HDL (mmol/L)	1.00 (0.32)	1.14 (0.38)	0.589	0.87 (0.32)	0.97 (0.21)	0.187	0.91 (0.29)	1.08 (0.22)	0.067
LDL (mmol/L)	1.94 (1.02)	2.56 (0.64)	0.316	1.42 (0.55)	2.60 (0.85)	<0.001***	1.90 (0.92)	2.60 (0.92)	0.109
TG (mmol/L)	1.12 (0.63)	1.09 (0.54)	0.700	0.81 (0.40)	1.37 (0.85)	0.016*	0.86 (0.36)	1.00 (0.56)	0.748

CGM – control group of men; IHDM – group of men with ischaemic heart disease; CC, CT, TT – genotypes; statistical significance *p < 0.05, **p < 0.01, ***p < 0.001

ence in the distribution of genotypes and alleles in the Uyghur and Han ethnic groups ($p > 0.05$). Genetic heterogeneity between populations, the size of the examined file may be responsible for the controversial results reported in scientific studies. It is well documented that a higher BMI is associated with a higher risk of cardiovascular disease (20). In our study, in a group of men, we found that, based on the WHO classification, men with CC and TT genotypes were in the overweight category (25–29.9 kg/m^2), and men with CT genotype were in the obese category (obesity class I). The mutant T allele was associated with higher BMI values in men in our study population. There are conflicting results regarding the association of body weight, BMI and cardiovascular mortality (21). While some studies have described an increased risk of death in overweight and obese men and women (22, 23); others have described an inverse relationship. In the female group, we found the following BMI categories: women with CC genotype (both groups), CT and TT genotypes (IHDW only) were categorized as obesity class I, and the control group of women with CT and TT genotypes were categorized as overweight. Relationship between overweight and obesity and cardiovascular mortality and all-cause mortality is commonly referred to as the "obesity paradox" (24, 25). Obesity is associated with elevated lipid levels, oxidative stress, and expression of inflammatory markers (26). Csige et al. (27) report that a 10 kg rise in body weight increases the risk of coronary heart disease by 12%, and also results in a 3 mmHg increase in systolic blood pressure, and a 2.3 mmHg increase in diastolic blood pressure. When we divided the study groups of men and women on the basis of BMI $\leq$ 24.9 kg/m^2 and BMI $\geq$ 25 kg/m^2, we detected no statistically significant difference in lipid profile parameters. Similar to the above-mentioned authors, we found statistical significance ($p = 0.028$) in the physiological parameter systolic blood pressure in the male group (control group 124.71 ± 7.11 mmHg; coronary heart disease group 137.06 ± 13.68 mmHg). The association between central obesity and changes in biochemical markers is currently debated because visceral fat produces adipocytes, which are directly associated with inflammatory processes and cardiometabolic complications. Based on our results, we can conclude that this scientific study contributes to other expert evidence reported by Garcez et al. and Rocha et al. (28, 29) because we also confirmed the correlations between BMI, waist circumference and biochemical markers such as TC, HDL and TG. However, it is not possible to make precise causal inferences because patients' biochemical markers may have been influenced by the medications taken. In the future, we propose to conduct a prospective cohort study to investigate other anthropometric and molecular genetic parameters to provide aspects of lifestyle, smoking and blood pressure in both sexes.

CONCLUSION

We conclude that a statistically significant ($p = 0.018$) possible association between the mutant T allele and coronary artery disease was detected in the male group in the rs2910829 polymorphism of the *PDE4D* gene. The genetic variant *PDE4D* rs2910829 may be a potential indicator of the level of cardiovascular risk. Men and women in the study cohort with CC, CT and TT genotypes belonged to the BMI classification of overweight and obesity class I. If we divided the observed groups of men and women on the basis of BMI $\leq$ 24.9 kg/m^2 and BMI $\geq$ 25 kg/m^2, we found statistical significance ($p = 0.028$) in the physiological parameter systolic blood pressure in the group of men. Body mass index, waist circumference measurements and biochemical markers of lipid profile may be considered as appropriate screening for obese individuals at risk of cardiovascular disease. Our results suggest that early intervention, correct treatment of persons with cardiovascular diseases (blood pressure $\geq$ 130/80 mmHg) with higher BMI can reduce morbidity and mortality from cardiovascular diseases in the Slovak population of both sexes. The genetic variant *PDE4D* rs2910829 may play a role in the pathogenesis of cardiovascular disease and may be a potential marker of cardiovascular risk. Since this is a pilot study in the Slovak population, we plan to validate the results in a larger cohort of individuals.

Conflicts of Interest

None declared

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