Cardiology Lett. 2023;32(3):169–174
doi:10.4149/Cardiol_2023_3_2

Correlation of the *PDE4D rs2910829* polymorphism genotypes with selected cardiovascular health indicators in the context of coronary heart disease

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Zigova M, Mydlarova Blascakova M, Petrejcikova E, Vaskova H, Hricova K. **Correlation of the *PDE4D rs2910829* polymorphism genotypes with selected cardiovascular health indicators in the context of coronary heart disease.** Cardiology Lett. 2023;32(3):169–174

Abstract. *Introduction:* The genetic variation of the protein-coding *PDE4D* gene may be a potential moderating factor of coronary risk. In this context, the aim of our research was to analyze correlation patterns between genotypes of the *PDE4D* gene variant and selected cardiovascular indicators in the eastern Slovakia case-control research group.

Design and methods: Overall, 230 individuals (130 CHD patients and 100 healthy controls) were analyzed for *PDE4D* gene *rs2910829* genotype and allele frequencies by the required protocols of the Real-Time PCR method. Genotyping data were correlated with values of selected anthropometrical and physiological indicators and levels of lipid profile markers.

Results: Genetic analysis confirmed the heterozygous genotype and mutant allele as the most frequent in our research group. The association between alleles and disease was not confirmed ($p > 0.05$). The results showed a statistically significant correlation between *rs2910829* genotypes and HDL cholesterol in CHD patients ($p < 0.05$) and a statistically significant correlation between genotypes and anthropometric indicators of hip circumference and BMI in controls ($p < 0.01$). A comparison of correlations between patients and controls confirmed statistically significant differences in 50% of cases.

Conclusion: Although the association between *rs2910829* and CHD was not confirmed, these results suggest that variations in the *PDE4D* gene may be correlated with some cardiovascular risk factors not only in CHD patients, but also in controls. The variant *rs2910829* may be a potential indicator of the cardiovascular risk, but this hypothesis should be confirmed in a larger sample of CHD patients and controls. Tab. 3, Ref. 19, on-line full text (Free, PDF) www.cardiologyletters.sk

Key words: *PDE4D* gene – *rs2910829* – coronary heart disease – correlation

Coronary heart disease (CHD) is a frequent heart condition with a confirmed impact on life expectancy. The disease rate is increasing around the world, including Slovakia. It is claimed that half of the Slovak population is at low risk for CHD and almost one-sixth is in the high-risk category. The current data may be even more

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Manuscript received 20 March 2023; accepted for publication 29 May 2023

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alarming and CHD – pathophysiological mechanisms are not fully elucidated (1-3).

Patients' disease heterogeneity is caused by multifactorial genetic and environmental etiologies and CHD risk factors tend to cluster and interact with each other. The estimation of the right combination of risk factors is relatively complicated. Previous studies have shown that coronary heart disease is the result of multiple causes, including variability in a lot of genes, for example in the *PDE4D* gene (4,5).

Genetic variation in the protein-coding *PDE4D* gene (phosphodiesterase 4D gene) is thought to be a potential moderator of coronary risk (6).

The aim of our research was to analyze correlation patterns between genotypes of the *PDE4D* gene variant and selected cardiovascular indicators in a group of CHD patients and healthy controls from eastern Slovakia.

Methods

Overall, 230 individuals (130 CHD patients and 100 healthy controls) were analyzed for the *PDE4D* gene, *rs2910829* (C/T). All research participants were informed about the aims and methods of the research and gave their informed consent. All institutional ethics requirements were met, and all procedures performed in the study were in accordance with the ethical standards of the research committee at the University of Prešov and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards (no. 6/2020 and 2/2013).

The main anthropometrical, clinical, and physiological information about patients and healthy controls was collected, along with blood samples for genetic analyses. The obtained data were statistically compared between CHD patients and controls by a Student t-test. Genomic DNA was extracted from peripheral blood samples using a conventional method (NucleoSpin® Blood; MACHEREY-NAGEL, Germany). The genotype and allele frequencies were established by the required protocols of the Real-Time PCR method (7500 Fast Real-Time PCR System; ABI, USA) and TaqMan chemistry (C___2820061_10).

After calculation of genotype and allele frequencies by counting method, genotype and allele frequency difference (χ^2 test), Hardy-Weinberg Equilibrium among cases and controls (HWE; χ^2 test), and the association between alleles and CHD were estimated by odds ratio with 95% confidence interval (OR, 95% CI). In the next step, genotyping data were correlated with the values of anthropometrical and physiological indicators (waist circumference, hip circumference, blood pressure, heart rate, BMI, and WHR index), levels of lipid profile markers (total cholesterol, LDL, and HDL cholesterols, triacylglycerols) (point-biserial correlation test; r_{pb}) and compared between analyzed groups (patients versus controls by r-z transformation). P-values under 0.05 determine statistical significance. All data were processed in MS Excel 365 and the online calculators available at *socscistatistics.com* were used for statistical analyses.

Results

In our research we analyzed anthropometrical, clinical, and physiological data obtained from 230 individuals. The research group was divided into CHD patients (56.52%) and controls (43.48%), with a mean age of 70.57 ± 9.10 and 64.38 ± 12.61 respectively. The mean values of baseline characteristics summarized in Table 1 were statistically compared. The significant differences were confirmed in the cases of age and the anthropometric variables like waist circumference, hip circumference, BMI, and WHR (**Table 1**). The CHD patients and controls significantly differ in the mean values of diastolic blood pressure, total cholesterol, and high-density lipoprotein ($p < 0.0001$).

In our research groups, the genotype distributions of *rs2910829* were in Hardy-Weinberg equilibrium (CHD patients: $\chi^2 = 0.32$, $p = 0.57$, and controls: $\chi^2 = 1.43$, $p = 0.27$). The pattern of genotype distribution was C/T > T/T > C/C in both subgroups. In comparison to the ancestral allele, the mutant allele of *rs2910829* was more frequent in CHD patients and controls, respectively. Our results did not confirm statistical differences in genotype and allele frequencies of *PDE4D rs2910829*. The presence of a mutant allele could be a potential risk factor (OR > 1), but statistical significance was not confirmed ($p > 0.05$) (**Table 2**).

Obtained genotyping data of *rs2910829* were correlated with values of anthropometrical and physiological

Table 1 Baseline characteristics of research subgroups

Variables	CHD patients (N = 130)		Controls (N = 100)		Statistics	
	Mean	± SD	Mean	± SD	t-test	p
Age (years)	70.57	9.10	64.38	12.61	-4.323	< 0.0001
Height (cm)	164.73	10.35	164.23	7.77	-0.403	0.6870
Weight (kg)	86.65	17.43	79.84	15.03	-3.116	0.0021
Waist circumference (cm)	114.07	15.30	99.34	14.54	-7.395	< 0.0001
Hip circumference (cm)	110.46	9.77	107.13	11.90	-2.330	0.0207
BMI (kg/m^2)	31.93	5.80	29.57	5.00	-3.245	0.0013
WHR	1.03	0.12	0.92	0.09	-7.657	< 0.0001
Systolic blood pressure (mmHg)	134.75	20.15	136.38	20.97	0.597	0.5508
Diastolic blood pressure (mmHg)	76.93	10.85	83.99	9.99	5.062	< 0.0001
Total cholesterol (mmol/l)	4.10	1.02	4.73	1.24	4.226	< 0.0001
HDL (mmol/l)	1.44	0.84	2.24	1.28	5.707	< 0.0001
LDL (mmol/l)	1.76	0.66	1.75	0.69	-0.112	0.9112
Triglycerides (mmol/l)	1.24	0.66	1.44	1.10	1.711	0.0884

BMI – Body mass index; CHD – coronary heart disease; HDL – high-density lipoprotein cholesterol; LDL – low-density lipoprotein cholesterol; N – number of individuals; SD – standard deviation; WHR – waist to hip ratio

indicators (waist circumference, hip circumference, BMI, WHR index, blood pressure, heart rate), and levels of lipid profile markers (total cholesterol, LDL, and HDL cholesterols, triglycerides) in CHD patients and controls, respectively (**Table 3**). A moderate negative and statistically significant correlation between *rs2910829* genotypes and HDL cholesterol was confirmed in CHD patients (p = 0.02). In controls, statistically significant results were confirmed only between polymorphism genotypes and anthropometric indicators of obesity: hip circumference (moderate negative correlation with p = 0.009) and BMI (moderate negative correlation with p = 0.008). A comparison of correlations between patients and controls confirmed statistically significant differences in 50% of cases (waist and hip circumferences, LDL cholesterol, triacylglycerols, and BMI).

Discussion

According to the OMIM database, the *PDE4D* gene (OMIM: 600129) is a protein-coding gene found on chromosome 5 (5q11.2-q12.1). The *PDE4D* gene is most abundantly expressed in skeletal muscle but also in most cell types involved in atherosclerosis (7,8). The gene product, cAMP-specific 3',5'-cyclic phosphodiesterase 4D, belongs to the class IV of cAMP-specific phosphodiesterases. In the cardiovascular system, the *PDE4D* gene

Table 2 Results of genetic analysis of *rs2910829* in research groups

Variant rs2910829	CHD patients (N = 130)	Controls (N = 100)
Homozygote reference (C/C)	24 (18.46%)	27 (27.00%)
Heterozygote (C/T)	60 (46.15%)	44 (44.00%)
Homozygote variant (T/T)	46 (35.38%)	29 (29.00%)
HWE	ns	ns
Genotype frequency difference	χ^2 = 2.6229, p = 0.2694	
Ancestral allele frequency (C)	41.54%	49.00%
Mutant allele (T)	58.46%	51.00%
Allele frequency difference	χ^2 = 2.5452, p = 0.1106	
Association	OR = 1.3522; 95% CI = 0.9330 to 1.9598; p = 0.1110	

CHD – coronary heart disease; CI – confidence interval; HWE – Hardy-Weinberg Equilibrium; N – number of individuals; ns – not significant result; OR – odds ratio

Table 3 Correlation analysis between *rs2910829* and selected cardiovascular health indicators

	CHD patients		Controls		r-z
Statistics	r_{pb}	**p-value**	r_{pb}	**p-value**	**p-value**
Waist circumference (cm)	0.0854	ns	-0.1781	ns	*
Hip circumference (cm)	-0.038	ns	**-0.2598**	**	*
BMI (kg/m²)	-0.0283	ns	**-0.2651**	**	*
WHR	0.1282	ns	-0.0513	ns	ns
Systolic blood pressure (mmHg)	0.0275	ns	-0.1044	ns	ns
Diastolic blood pressure (mmHg)	0.068	ns	-0.0013	ns	ns
Total cholesterol (mmol/l)	-0.156	ns	-0.1387	ns	ns
HDL (mmol/l)	**-0.2032**	*	-0.1068	ns	ns
LDL (mmol/l)	0.0463	ns	0.0754	ns	*
Triglycerides (mmol/l)	-0.1366	ns	0.0899	ns	*

BMI – Body mass index; CHD – coronary heart disease; HDL – high-density lipoprotein cholesterol (mmol/l); LDL – low-density lipoprotein cholesterol (mmol/l); ns – not significant result; r_{pb} – point-biserial correlation; r-z – r to z transformation; WHR – Waist to hip ratio; * – p-value ≤ 0.05; ** – p-value ≤ 0.01

product acts as a signal transduction molecule, which is responsible for sarcoplasmic reticulum Ca^{2+} release and cardiac contractility (7,9). The analysis of *rs2910829*, which is an intronic variant of the *PDE4D* gene, has been the subject of several studies (10-12). It is assumed that the *rs2910829* polymorphism C allele is implicated in multiple health complications. In the study of *Shao et al.,* allele C increased the risk of CHD and the incidence of stroke (10). The association of *PDE4D* gene variants and the risk of ischemic stroke was analyzed in different study groups, for example, in Taiwanese and South Indian populations (13, 14). The allele and genotype frequencies of the *rs2910829* polymorphism were also investigated in the cerebral infarction group (11). Data about the association of *rs2910829* with coronary heart disease or another cardiovascular or cerebrovascular complication are insufficient or controversial, but it is believed that the *PDE4D* gene and its genetic variants may have a role in modulating the atherogenic process in the carotid arteries (6,12-16). For example, no significant risk associated with CHD was evaluated by *Sinha et al.* in the context of two *PDE4D* gene polymorphisms, including *rs2910829* (6). The results of *Yoon et al.* meta-analysis showed a nonsignificant association with ischemic stroke (17). In our study, the association of *rs2910829* with coronary heart disease in research subgroups of 230 individuals was not statistically confirmed (OR = 1.3522, p = 0.1110). After comparison in our case-control research group, the genotype and allele frequencies did not differ significantly and showed a predominance of genotypes composed of one or two mutant alleles T. The allele C was presented at a lower frequency than the mutant allele T, which was confirmed in 58.46% of CHD patients and in 51% of controls. In the *Ma et al.* study, the C allele frequency of the *rs2910829* in the group of cerebral infarction patients was significantly higher than in the control group (81.0% vs. 76.4%, p = 0.031). Similar to our research, the authors conclude that there was no statistically significant difference in the genotype frequencies (χ^2 = 5.765, p = 0.056). On the other hand, their results also indicated that C allele carriers were at a higher risk of cerebral infarction (OR value of 1.314, p < 0.05) (11).

The main goal of our study was to analyze the correlation pattern of the *PDE4D rs2910829* polymorphism genotypes with selected cardiovascular health indicators in the context of coronary heart disease. There is very little information available from this area. In the study of *Rodríguez-Pérez et al.*, *PDE4D* polymorphisms were associated with the presence of some clinical and metabolic parameters like central obesity, hypertriglyceridemia and increased levels of markers: aspartate transaminase (ALT >p75), lipoprotein (a) (Lp (a) >30 mg/dL), thrombin–antithrombin complex (TAT > p75), fatty liver, and vitamin D (values < 30 ng/dL) (18). In another study, the results showed a significant association of *rs2910829* with one of the established risk factors for CHD, which was high serum triglycerides (p ≤ 0.05) (6).

Our results of correlation analysis confirmed a moderate negative and statistically significant correlation

between *rs2910829* genotypes and HDL cholesterol in CHD patients (p = 0.02), which points to the influence of genotypes of *rs2910829* with mutant alleles on decreasing values of the protective HDL cholesterol. This may be a potential mechanism for increased cardiovascular risk in our CHD patients because HDL levels are an independent CHD risk factor (19). In controls, mostly negative correlations were confirmed, and statistically significant results were detected between polymorphism and anthropometric indicators of hip circumference (moderate negative correlation with p = 0.009) and BMI (moderate negative correlation with p = 0.008). The presence of the mutated allele of *rs2910829* in the controls could potentially have a protective effect, but our findings need to be verified in a larger group. A comparison of correlations between patients and controls confirmed statistically significant differences in 50% of cases (waist and hip circumferences, LDL cholesterol, triglycerides, and BMI).

Conclusion

Our study reassured us that coronary heart disease is a multifactorial and polygenic disease. Although the significant association between *rs2910829* and CHD was not confirmed, our findings suggest that variations in the *PDE4D* gene may be correlated with decreasing HDL levels and could indirectly increase the risk of cardiovascular complications in patients with CHD. The *PDE4D* genetic variant *rs2910829* may be a potential indicator of the level of cardiovascular risk, but this hypothesis should be confirmed in a larger sample of CHD patients and controls. Our results showed that it is necessary to approach the disease comprehensively.

Acknowledgements

The research was supported by an independent research grant provided by Pfizer (ID 56862787) and MŠVVaŠ SR – KEGA no. 002PU-4/2021.

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