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ORIGINAL ARTICLE

POLYMORPHIC VARIANT RS11206510 IN PCSK9 AND RISK OF CORONARY ARTERY DISEASE IN BULGARIANS

R. Tzveova[1], T. Yaneva-Sirakova[2], G. Naydenova[3], S. Vandeva[4], D. Pendicheva-Duhlenska[5], P. Atanasov[6], V. Mitev[7], R. Kaneva[7]

[1]Department of General and Clinical Pathology, University Hospital "Tsaritsa Yoanna – ISUL" – Sofia, Bulgaria
[2]Acibadem City Clinic Cardiovascular Center – Sofia, Bulgaria
[3]Department "Propaedeutics of Internal Diseases", Medical University – Pleven, Bulgaria
[4]Clinical Center of Endocrinology and Gerontology, Medical University – Sofia, Bulgaria
[5]Department of Pharmacology and Toxicology, Medical University – Pleven, Bulgaria
[6]National Sports Academy – Sofia, Bulgaria
[7]Molecular Medicine Center, Department of Medical Chemistry and Biochemistry, Medical University – Sofia, Bulgaria

Abstract. Objective: *The aim of this study was to investigate the potential association of rs11206510 in PCSK9 gene with coronary artery disease (CAD) and myocardial infarction (MI) in Bulgarians.* ***Materials and Methods:*** *The current analysis included 261 patients with angiographically documented CAD (153 with MI and 108 without MI) and 496 population – based controls. Genomic DNA was extracted from venous blood samples. The selected polymorphism was genotyped by TaqMan SNP Genotyping Assay. The genotype and allele frequencies were compared between cases and controls using χ^2 test.* ***Results:*** *In this study, the presence of the T allele of rs11206510 in the PCSK9 gene was found to be associated with elevated risk for MI in patients with already existing myocardial ischemia (allele T, OR1.78,CI95:1.16-2.73, p = 0.007). The result was enhanced in the male subgroup (allele T, OR1.74, CI95:1.02-2.96, p = 0.038). Also, we found reduced risk of CAD (without MI) for T allele (OR0.70, CI95:0.49-0.99, p = 0.04). This trend was stronger in the male subgroup (OR0.56, CI95:0.35-0.90, p = 0.02). There was not any relationship of the studied genetic variant with the levels of total cholesterol, triglycerides, low density lipoproteins and high-density lipoproteins, or with systolic and diastolic blood pressure values.* ***Conclusion:*** *Our study found a difference in the frequencies of rs11206510 genotypes and alleles in the PCSK9 gene between cases and controls, and the relationship of the investigated polymorphism to the risk of cardiac injury in the Bulgarian population was demonstrated. Further investigations with a larger number of cases and controls will be needed in order to evaluate a possible association between this variant and CAD/MI in Bulgarians.*

Key words: *PCSK9, polymorphic variant, coronary artery disease, Bulgarians*

Corresponding author: *Reni Tzveova, Department of General and Clinical Pathology, University Hospital "Tsaritsa Yoanna – ISUL", 1527 Sofia, Bulgaria, 8 Byalo more Street, e-mail: renitzveova@abv.bg*

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INTRODUCTION

The gene PCSK9 codes the protein proprotein convertase subtilisin/kexin type 9. The enzyme reduces the number of low-density lipoprotein receptors (LDLR) at the surface of the hepatocyte [1]. This is followed by changes in lipid profile and elevated levels of LDL cholesterol in the blood. In 2003 year the first mutation in the PCSK9 gene was found - dominant form of familial hypercholesterolemia, and this initiated the understanding of the cholesterol metabolism [2].

A meta-analysis of 32 scientific studies showed significant association between the allele variant G of rs505151 in PCSK9 and higher levels of LDL cholesterol in the serum of Caucasian people. The same polymorphism was also associated with higher incidence of cardio-vascular incidents. On the other hand, the T allele of variant rs11591147 in PCSK9 was associated with lower levels of serum cholesterol and LDL cholesterol, as well as with lower risk for cardio-vascular incidents [3].

Other meta-analyses show association between the variant rs505151 in PCSK9 with higher levels of cholesterol, LDL-cholesterol, and cardio-vascular risk [4, 5].

These variants, as well as others in the gene PCSK9, can be used as genetic biomarkers for evaluation of cardio-vascular risk. This will help the diagnostic and prognostic process and will enhance the success of treatment in cardio-vascular diseases associated with dyslipidemia. That is why the indebt study of the polymorphisms of PCSK9 in the Bulgarian population is necessary in the context of successful future genetically based prophylaxis of socially important diseases.

MATERIALS AND METHODS

The study included patients with angiographically proven coronary artery disease (CAD), with or without myocardial infarction, who were hospitalised in one of the affiliated cardiology clinics. All population controls were from the DNA biobank of the Molecular Medicine Center, Medical University, Sofia, Bulgaria. The National University Complex for Biomedical and Translational Research (NUCBTR) is a strategic network of infrastructures for fundamental and translational biomedical research and includes partners from two of the largest medical universities in Bulgaria, the Medical University of Sofia and the Medical University of Plovdiv, as well as a number of hospital and research centers. At the core of the University Complex is the unification of the largest biobanks for the storage of biological material and clinical data in the country.

Inclusion criteria for the patients with CAD: 1. Age between 18 and 75 years; 2. Systolic blood pressure ≤ 120 mm Hg; 3. Diastolic blood pressure ≤ 80 mm Hg; 4. CAD proven by coronary angiography; 5. Hospitalized or ambulatory patients. 6. Signed written informed consent for participation in the current study.

Exclusion criteria: 1. Age below 18 years or above 75 years; 2. Absence of CAD established by coronary angiography; 3. Absence of signed informed consent; 4. Acute renal failure; acute liver failure; 5. Severe anemia, requiring transfusion; 6. Chronic dialysis; 7. Epilepsy.

The demographic and clinical characteristics of the studied groups of patients with CAD with and without MI and the population controls, are given in Table 1.

The basic laboratory panel recommended by the World Health Organization for the precise assessment of cardiovascular risk (serum glucose levels, total cholesterol, ASAT, ALAT, GGT, IGF-1 – insulin like growth factor 1, TSH – thyroid stimulating hormone) known only for 1/3 of the population controls and for all patients with CAD.

All participants in the study were genotyped for polymorphic variant rs11206510 in gene PCSK9 (Table 2). The

Table 1. Demographic and clinical characteristics of the studied groups of patients and the population controls

	CAD (N = 108)	MI (N = 153)	Population controls (N = 496)
Age (years)	66.27 ± 8.81	66.34 ± 10.39	36.08 ± 12.99
Sex (males)	60 (55.56)	92 (60.53)	241 (48.59)
BMI (kg/m2)	29.66 ± 5.72	28.36 ± 4.89	25.66 ± 4.91
Total cholesterol (mmol/l)	5.64 ± 1.04	5.94 ± 0.75	4.99 ± 0.94
Triglycerides (mmol/l)	1.32 ± 0.67	2.07 ± 0.56	0.93 ± 0.56
LDL-cholesterol (mmol/l)	4.23 ± 1.06	4.14 ± 0.79	3.18 ± 0.88
HDL-cholesterol (mmol/l)	1.22 ± 0.37	1.39 ± 0.28	1.62 ± 0.40
Systolic blood pressure (SBP) (mmHg)	147.86 ± 21.83	135.92 ± 10.96	-
Diastolic blood pressure (DBP) (mmHg)	87.50 ± 13.64	83.64 ± 6.60	-

Table 2. Genome information for the polymorphic variant rs11206510 in gene PCSK9

Chromosome	Position	Gene	Identification number	Change	Type of change	Localization
1p32.3	55496039	PCSK9	rs11206510	T > C	transition	intron

genomic DNA was isolated from peripheral venous blood. The studied polymorphic variant was genotyped with the use of TaqMan SNP Genotyping Assay (Applied Biosystems).

The comparative statistical analysis of the genotypic and allelic frequencies between patients with CAD and healthy controls was done with the use of $\chi 2$ test. We also conducted a check of the Hardy – Weinberg equation. For statistically significant difference was accepted $p < 0.05$. The analysis was done with SPSS version 19.0 (IBM).

Ethical Aspects: the study was approved by the Ethical committee of Medical University Sofia, Bulgaria and the Ethical Committee of Medical University Pleven. The study was conducted in accordance with ethical principles of the Declaration of Helsinki for human rights form 1975 year.

RESULTS

The current analysis included 261 patients with angiographically proven CAD (153 patients with MI [6, 7] and 108 without MI) and 496 population controls. All participants were genotyped for the polymorphic variant rs11206510 in gene PCSK9.

The allelic and genotypic frequencies of rs11206510 in gene PCSK9 in the group of patients with CAS and the population group of Bulgarians is given in tables 3-6.

As a result of the current genetic analysis we found, that in Bulgarians with CAD without MI, the more frequent T allele of the polymorphic variant rs11206510 in gene PCSK9 could not be associated with higher risk for CAD incidence (OR 0.70, CI95: 0.49-0.99, p = 0.04). This tendency was stronger for males as compared to females (OR 0.56, CI95:0.35-0.90, p = 0.02) (Table 4).

The polymorphic allele T was a risk one for the incidence of MI in patients with already developed CAD – allele T, OR 1.78, CI95: 1.16-2.73, p = 0.007. This effect was stronger for males as compared to females – allele T, OR 1.74, CI95: 1.02-2.96, p = 0.038 (Table 6).

As addition, we conducted associative analysis for a potential association between several clinical variables and a certain genotype of rs11206510 in gene PCSK9 (Table 7).

Table 3. Allelic and genotypic distribution of the studied polymorphic variant rs11206510 in gene PCSK9 in the group of patients with CAD (with or without MI) and the population controls in Bulgarians

Chr	Gene	Variant	Model	Genotype/ allele	all			Males			Females		
					CAD n (%)	controls n (%)	P	CAD n (%)	Controls n (%)	P	CAD n (%)	Controls n (%)	P
1	PCSK9	rs11206510	genotypic	CC	8 (2.49)	15 (3.02)	0.77	4 (1.95)	5 (2.07)	0.56	4 (3.45)	10 (3.92)	0.58
				CT	87 (27.10)	125 (25.20)		59 (28.78)	54 (22.41)		28 (24.14)	71 (27.84)	
				TT	226 (70.41)	356 (71.78)		142 (69.27)	182 (75.52)		84 (72.41)	174 (68.24)	
			allelic	C	103 (16.04)	155 (15.63)	T 0.82 (OR 0.97, CI95:0.74-1.27)	67 (16.34)	64 (13.28)	T 0.20 (OR 0.78, CI95:0.54-1.14)	36 (15.52)	91 (17.84)	T 0.44 (OR 1.18, CI95:0.78-1.80)
				T	539 (83.96)	837 (84.37)		343 (83.64)	418 (86.72)		196 (84.48)	419 (82.16)	

Table 4. Distribution of the allelic and genotype frequencies of the studied polymorphic variant rs11206510 in gene PCSK9 in the group of patients with CAD without MI and population controls

Chr	Gene	Variant	Model	Genotypic/allelic	All			Males			Females		
					CAD n (%)	Controls n (%)	P	CAD n (%)	Controls n (%)	P	CAD n (%)	Controls n (%)	P
1	PCSK9	rs11206510	Genotypic	CC	5 (4.03)	15 (3.02)	0.11	3 (4.00)	5 (2.07)	0.03	2 (4.08)	10 (3.92)	0.76
				CT	42 (33.87)	125 (25.20)		26 (34.67)	54 (22.41)		16 (32.65)	71 (27.84)	
				TT	77 (62.10)	356 (71.78)		46 (61.33)	182 (75.52)		31 (63.27)	174 (68.24)	
			allelic	C	52 (20.97)	155 (15.63)	T 0.04 (OR 0.70, CI95:0.49-0.99)	32 (21.33)	64 (13.28)	T 0.02 (OR 0.56, CI95:0.35-0.90)	20 (20.41)	91 (17.84)	T 0.57 (OR 0.84, CI95:0.49-1.46)
				T	196 (79.03)	837 (84.37)		118 (78.67)	418 (86.72)		78 (79.59)	419 (82.16)	

Table 5. Distribution of the allelic and genotype frequencies of the studied polymorphic variant rs11206510 in gene PCSK9 in the group of patients with CAD with MI and population controls

Chr	Gene	Variant	Model	Genotypic/allelic	All			Males			Females		
					CAD n (%)	Controls n (%)	P	CAD n (%)	Controls n (%)	P	CAD n (%)	Controls n (%)	P
1	PCSK9	rs11206510	genotypic	CC	3 (1.52)	15 (3.02)	0.46	1 (0.77)	5 (2.07)	1	2 (2.99)	10 (3.92)	0.26
				CT	45 (22.84)	125 (25.20)		33 (25.38)	54 (22.41)		12 (17.91)	71 (27.84)	
				TT	149 (75.64)	356 (71.78)		96 (73.85)	182 (75.52)		53 (79.10)	174 (68.24)	
			allelic	C	51 (12.94)	155 (15.63)	T 0.21 (OR 1.24, CI95:0.89-1.75)	35 (13.46)	64 (13.28)	T 1.00 (OR 0.98, CI95:0.63-1.53)	16 (11.94)	91 (17.84)	T 0.10 (OR 1.60, CI95:0.91-2.83)
				T	343 (77.06)	837 (84.37)		225 (86.54)	418 (86.72)		118 (88.06)	419 (82.16)	

Table 6. Distribution of the allelic and genotype frequencies of the studied polymorphic variant rs11206510 in gene PCSK9 between the groups of patients with CAD with and without MI

Chr	Gene	Variant	Model	Genotypic/allelic	All			Males			Females		
					With MI n (%)	Without MI n (%)	P	With MI n (%)	Without MI n (%)	P	With CAD n (%)	Without n (%)	P
1	PCSK9	rs11206510	genotypic	CC	3 (1.52)	5 (4.03)	0.01	1 (0.77)	3 (4.00)	0.08	2 (2.99)	2 (4.08)	0.21
				CT	45 (22.84)	42 (33.87)		33 (25.38)	26 (34.67)		12 (17.91)	16 (32.65)	
				TT	149 (75.64)	77 (62.10)		96 (73.85)	46 (61.33)		53 (79.10)	31 (63.27)	
			allelic	C	51 (12.94)	52 (21.0)	T 0.007 OR 1.78 (CI95: 1.16-2.73)	35 (13.46)	32 (21.33)	T 0.038 (OR 1.74, CI95: 1.02-2.96)	16 (11.94)	20 (20.41)	T 0.08 (OR 1.89, CI95:0.92-3.87)
				T	343 (87.06)	196 (79.0)		225 (86.54)	118 (78.67)		118 (88.06)	78 (79.59)	

Table 7. Associative analysis for potential association between the given clinical variables, blood pressure values, and a certain genotype of rs11206510 in gene in patient with CAD

Genotype		Total cholesterol (mmol/L)	HDL-cholesterol (mmol/L)	LDL-cholesterol mmol/L)	TGs (mmol/L)	SBP (mmHg)	DBP (mmHg)
CC	mean	5.26	1.46	3.46	1.69	130.00	80.00
	Standard Deviation (± SD)	0.997	0.307	1.033	0.891	14.032	5.742
	minimum	4.00	1.00	3.00	1.00	130.00	80.00
	maximum	7.00	2.00	5.00	3.00	13.00	80.00
	Standard error (± SE)	0.446	0.137	0.462	0.398	3.523	2.132
CT	mean	5.22	1.46	3.41	1.44	154.55	87.73
	Standard Deviation (± SD)	0.834	0.357	0.795	0.772	15.076	6.842
	minimum	3.00	1.00	2.00	1.00	140.00	80.00
	maximum	7.00	2.00	5.00	3.00	180.00	100.00
	Standard error (± SE)	0.101	0.044	0.096	0.094	4.545	2.063
TT	mean	5.39	1.41	3.54	1.54	142.41	85.93
	Standard Deviation (± SD)	1.139	0.403	1.150	0.905	20.493	14.280
	minimum	3.00	1.00	2.00	2.00	90.00	60.00
	maximum	11.00	3.00	8.00	5.00	200.00	130.00
	Standard error (± SE)	0.076	0.027	0.078	0.061	3.944	2.748
P		0.509	0.586	0.667	0.647	0.163	0.815

We could not find any statistically significant difference between the mean values of total cholesterol value, HDL-cholesterol (HDL), LDL-cholesterol (LDL), triglyceride levels (TGs), systolic blood pressure (SBP), diastolic blood pressure (DBP) in the groups of patients with and without the genetic variants CC, CT and TT of rs11206510 in PCSK9 (Table 7).

DISCUSSION

Dyslipidemia is one of the major risk factors for development and progression of cardio-vascular diseases [8]. The statins play a basic role in the treatment of dyslipidemia, however, they may have different clinical and laboratory side effects with respect to the genetic profile of the given patient. The finding of mutations in the PCSK9 gene recently, and its association with the levels of LDL-cholesterol was related to cardio-vascular incidents. The gene PCSK9 encodes an enzyme, which reduces LDLR on the hepatocyte membrane. This induces changes in the lipid profile and elevation of LDL-cholesterol [2]. The polymorphic variants of PCSK9 have a complex significance for the pathogenesis and regulation of LDL levels [2, 9]. This induced the development of a whole new drug class for the treatment of severe dyslipidemia [9, 10].

A multilocus study, aimed at assessment of the genetic risk on the basis of 27 chromosome loci, incl. PCSK9, identified individuals with elevated risk for cardio-vascular events. These findings were based on the results from the Malmo Diet and Cancer study, and four additional randomized controlled trials: JUPITER, ASCOT, CARE and PROVE IT-TIMI 22 [11].

The aim of the current trial was to test if there was association between polymorphic variant rs11206510 in gene PCSK9 and the risk for CAD and MI in Bulgarians. We did not find T allele associated with a higher risk for CAD, but in patients with already developed ischemia, it was associated with elevated risk for MI.

A possible explanation for the discrepancy with the literature because of lack of association of T allele and CAD can be explained with two factors, which can be corrected in future trials. First, we used population controls and not age-matched control individuals. It can be suspected that a large number of genetically predisposed but clinically still not manifested young individuals were included in the control group. Second, the patients with angiographically proven CAD in the study were relatively elderly, with multiple concomitant cardio-vascular and socio-economic risk factors that may modify the added, non-genetically associated cardio-vascular risk. The contemporary conception for the genetic predisposition of CAD is that it is based on multiple genes and loci with relatively small individual significance [12]. In a relatively small sample size, some discrepancies with the findings from GWAS may arise. However, it is important to emphasize that the study of the genetic aspects of CAD in Bulgarians is important, because this is a high-risk group and specific genetic variants may potentially play a role. It should also be noted, that a variety of modifiable risk factors that form the environment could influence the genetic expression of certain variants and further modify the local phenotypic spectrum [13, 14].

It was impossible from a practical point of view to find a control group of healthy individuals with a mean age, corresponding to the mean age of the CAD patients. Because of this reason, in the present study we recruited a twice larger sample of population controls compared with patients with cardiovascular disease. Their DNA was from the national biobank of the Molecular Medical Center, Medical University Sofia, Bulgaria. The use of a much larger control group was meant to match the polymorphic variant frequencies of the general population. It was expected that the frequencies in the patients' group would be much higher.

In the literature, the precise association between a given allele/genotype is not one. In a study of Zhu et al. was found that CC and CT genotypes were associated with elevated CAD mortality rate in patients on statins [15].

The result for a higher risk for MI in T allele corresponded to the major findings in the literature [16].

The difference in the plasma LDL levels and the total cholesterol levels did not differ significantly between the wild type and the heterozygous to the polymorphic allele C. The potential explanations were two: either the sample size is not big enough, or the effect of statins treatment masks the precise genetic association with a given levels of cholesterol. According to the general recommendations, all the patients with CAD have indications for statin treatment [17, 18, 19]. In the literature there are many positive results for a potential association of rs11206510 in gene PCSK9 with the values of lipids in blood. There are studies in the literature for a potential association with the given polymorphism and the risk for CAD.

A meta-analysis from 2013 of Zhou et al showed that the polymorphic variant was a risk factor for CAD in

Caucasians (P = 0.007, OR = 1.09, 95% CI = 1.03-1.17), but not in Asians (P = 0.167, OR = 1.16, 95% CI = 0.94-1.43) [20]. Earlier studies in the field proved the role of the TGs, LDL cholesterol, HDL cholesterol as independent predictors for CAD. Some genetic variants may be exposed to the influence of serum lipids and their expression thus, modified [22].

In 2010, Teslovich et al. published association study of case-control type, that included more than 100,000 Europeans. They test the association between cardiovascular disease and several polymorphic variants in genes PCSK9 and LDLR [23]. The results showed that the alleles rs11206510-T in gene PCSK9 and rs1122608-G in gene LDLR were associated with higher levels of LDL cholesterol and total cholesterol, and thus were risk factors for cardio-vascular disease.

Guella et al. in 2010 year found that the rare C allele of rs11206510 in gene PCSK9 significantly correlated with lower LDL levels – OR = 0.82, 95% CI = 0.73-0.93, P = 1.89×10-3) and the total cholesterol (OR = 0.80, 95% CI = 0.72-0.89, P = 8.12×10-5) in Italians [24].

According to Willer et al., rs11206510 was positively associated both with the concentration of LDL cholesterol and the risk for CAD in Europeans [25].

Another three genome association studies confirmed the significance of rs11206510 (T > C) in PCSK9 as a risk factor for CAD in Europeans [26-28].

Contrary to the above results were the findings of Reilly et al in 2011 year, who could not confirm such an association in Europeans in a genome wide study [29].

CONCLUSION

This is the first study of the potential association of polymorphic variant rs11206510 in gene PCSK9 and CAD with or without MI in Bulgarians. The data cannot confirm the role of this polymorphism in cardio-vascular pathology because of the small effect on the studied disease and the possibly insufficient number of patients and controls. The further study of other polymorphic variants in PCSK9 will help build the whole picture for the possible role of this gene in the cardio-vascular pathology, locally in Bulgarians.

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