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ORIGINAL ARTICLE

THE ROLE OF CERTAIN POLYMORPHIC VARIANTS IN GENES, PREVIOUSLY ASSOCIATED WITH BLOOD PRESSURE VALUES, WITH REFERENCE TO THE RISK OF DEVELOPMENT OF CORONARY ARTERY DISEASE

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Abstract. *The aim of the study was to analyze the effect of polymorphic variants previously associated with arterial hypertension (AH) in Genome Wide Association Studies (GWASs) in/next to genes and locuses CYP7A1 and PLEKHA7 on the development of coronary artery disease (CAD) in Bulgarian patients. A hundred and nine consecutive patients with angiographically documented CAD were studied. The genotyping was done with 7900 HT Fast Real-Time PCR (Applied Biosystems) with TaqMan® method. The control group consisted of 192 healthy population controls, selected from the biobank of the Molecular Medicine Center. SPSS and PLINK were used for the statistical analysis with level of significance < 0.05 and confidence interval 95%. The mean age of the studied patients was 63.71 ± 9.35 years; 35 (35%) females. Previous myocardial infarction (MI) had 38(38%); one-vessel – 39 (39%); two-vessel – 28 (28%); three-vessel disease – 34 (34%); 43 (43%) were with diabetes mellitus; 92 (92%) – with arterial hypertension (AH); 77 (77%) – with dyslipidemia; 42 (42%) were smokers; 25 (25%) were obese. We did not find any significant association between CAD and polymorphism rs11191548 near CYP17A1 and only a tendency for genotype of rs381815 in PLEKHA7 (p = 0.06; OR 0.64; CI 0.40-1.02 for CAD) under dominant model. This is of practical importance both for studying the genetic aspects of CAD in the future and for enlargement of the current database.*

Key words: *coronary artery disease, PLEKHA7, CYP17A1, steroid hormones, Ca2+ exchange, arterial hypertension*

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INTRODUCTION

Cardio-vascular diseases are multifactorial conditions. Their clinical manifestation depends on the interaction between environmental and multiple genetic factors. Coronary artery disease (CAD) remains the leading mortality cause in the developed countries, despite the innovative and effective treatment methods.

In previous studies, *rs11191548* next to *CYP17A1* gene and *rs381815* in *PLEKHA7* gene were associated with arterial hypertension (AH). Both polymorphisms were also involved in several processes that could be associated with changes in large and small vessels, and secondarily – with atherosclerosis.

The *CYP17A1* gene is localized on chromosome 10q24.3 and expressed primarily in the suprarenal glands and the gonads. It encodes one of the members of the P450c17 family. The proteins, which are produced from its transcription, are with 17α-hydroxylase and 17,20 – lyase activity. They have a major role in steroid (including sex hormones) synthesis [1]. These are monooxygenases, which are found in the endoplasmic reticulum. Mutations in the gene can lead to reduced androgenic production. The association between CAD and androgens was documented in previous studies [2]. Lower than normal testosterone levels were correlated with higher CAD morbidity [3]. Testosterone is involved in the lipoprotein metabolism and its high levels are associated with elevated concentration of high-density lipoprotein cholesterol (HDL) and low level of triglycerides (TG), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and total cholesterol (TC) [4]. Another potential mechanism for the role of androgens and particularly testosterone in CAD is the potentiation of endothelial dysfunction via low nitric oxide (NO) secretion [5]. Testosterone also impairs the coagulation cascade by elevation of tissue plasminogen activator expression and blockage of its inhibitor [6]. Lower testosterone levels are associated with higher concentrations of factor VIII (fVIII), endothelial dysfunction and vascular inflammation [6].

PLEKHA7 protein (Plekstrin homology domain containing family A member 7) is involved in the PDZD11 protein complex that attaches nectins to cadherin and connects to the cytoskeleton protein complex *PLEKHA7*. This leads to the stabilization of nectins to adherens junction. It attaches the microtubules to adherent junctions and ensures their integrity [7]. This mechanism is involved also in Ca2+ regulation [8]. Goellner et al. [9] found that *PLEKHA7* correlates functionally with PDZD11, and thus affects membrane Ca2+ ATPase and controls calcium homeostasis. The connection of *PLEKHA7* with p120 and afadin - E-cadherin complex maintains the integrity of adherent junctions of endothelial cells [10]. Consequently, *PLEKHA7* plays significant role in the structural and functional integrity of the endothelium. Changes of its expression may play a role in atherogenesis.

MATERIALS AND METHODS

We included 109 consecutive patients with angiographically documented CAD. They were with either early atherosclerosis or with family history for early atherosclerosis. The group was genetically tested and full medical risk factors history was available for 100 of them (descriptive statistics given is with valid percentage). The study was conducted in accordance with the Ethical recommendations of the Helsinki Declaration from 1975 and after approval from the local Ethical committee of Medical University of Sofia. All patients signed written informed consent for participation in a genetic study. The control group consisted of 192 healthy population controls selected from the biobank of Molecular Medical Center, Medical University of Sofia, Bulgaria.

Medical history and physical examination

Full medical history was gathered for every patient with a special emphasis on cardio-vascular risk factors, family history, and concomitant target organ damage.

Laboratory tests

A laboratory test minimum was required for inclusion in the study and for diagnosis of the concomitant cardio-vascular risk factors.

Instrumental evaluation

Echocardiography, ECG, Angiographic Analysis – Quantitative angiographic analyses were performed using freely available software (Dicom Works version 3.1.5b, Paris, http://www.dicomworks.com/). Catheter calibration was used in all cases. Bifurcation lesions were classified according to the Medina classification using an index of 1 for stenosis greater than 50% and an index of 0 for no stenosis. The lesions with ostial significant stenosis of SB (percentage diameter stenosis > 50%) were classified as true bifurcation lesions. The changes of SB percentage diameter stenosis (SB%DS) before procedure, after stenting, and at the end of PCI were assessed.

Genetic testing

Blood sample was collected in a K3EDTA tube from every studied patient. Most DNA samples were isolated by the salting out Miller method [11]. For the healthy controls' we used CHEMAGEN® Magnetic Separation

Station (Perkin Elmer). The DNA isolation from peripheral blood with the semiautomated separating system Chemagen is based on the use of magnetic particles in electromagnetic field. The concentration of the isolated DNA was assessed with Spectrophotometer Nanodrop ND1000. Automated liquid handling BIOMEK®FXp system (Beckman Coulter) was used for dispensing DNAs and PCR reaction set up. The screening for the selected polymorphisms in the genes was done on a HT7900 Fast RT-PCR system with TaqMan™-assays (Applied Biosystems). The selected SNPs with their location and type are listed in Table 1.

Table 1. The selected polymorphic variants in the current study with their location and type.

Chromosome	Position	Gene	Variant	Type
10	103086421	*CYP17A1*	rs11191548	3 prime UTR
11	16880721	*PLEKH7*	rs381815	intron

The genotype and allele frequencies of studied polymorphisms were analysed after checking for deviation from Hardy Weinberg equilibrium with PLINK software – PLINK v. 1.9 (Purcell et al., 2007).

Statistical analysis

For the purposes of statistical analysis, we used SPSS v. 19 (IBM). Quantitative data was given as a mean and SD, categorical data – as frequencies. Descriptive statistics, correlation analysis, χ^2, comparison of the distribution of the allelic and genotypic frequencies were performed with level of significance 0.05.

RESULTS

Characteristics of the studied group

The mean age of the studied patient group was 63.71 ± 9.35 years (minimal 32 maximal 84 years). All patients above the age of 70 years upon recruitment were with family history and anamnesis for early first manifestation of atherosclerosis (before age of 55 for men and before 65 for women). The males were 35 (35%). There was a significant difference between the mean age of women 67.03 ± 7.98 years and the men 61.92 ± 9.6 years (p = 0.009, CI 95%, -8.88; -1.33). The patients with previous MI were 38 (38%), single vessel disease had 39 (39%), two-vessel disease 28 (28%), multi-vessel disease 34 (34%). We used the current definition for CAD [12] and the third definition for MI [13]. Diabetes mellitus had 43 (43%) of the patients [14]. AH had 92 (92%), as of the current European guidelines around the time of the termination of the study 15] dyslipidemia had 77 (77%) [16], 42 (42%) were smokers and 25 (25%) were with obesity. The mean blood pressure values for the studied group were around the target level [15] 135.21 ± 17.44 mm Hg for the systolic and 82.42 ± 8.46 mm Hg for the diastolic. Concomitant cardiovascular diseases: 23 (23%) of the patients had cerebrovascular disease; 15 (15%) had a history of previous stroke; 18 (18%) were with peripheral artery disease. Echocardiography revealed that 75 (75%) of the patients were with left ventricular hypertrophy, the mean ejection fraction was lower than normal 52.58 ± 12.96. The mean number of stents per patient was 2.5 ± 2.27, maximal 9. Twenty six of the patients had balloon dilatation after positive FFR testing for functional significance of intermediate stenosis or had angiographically/functionally proven small-vessel disease (CFR) [17,18]. The majority of patients had a mean of 4 stents (19%). The relative percent of patients taking a certain medication is shown in Figure 1.

Distribution of allele and genotype frequencies of the studied polymorphisms

The genotype distribution of the studied polymorphisms in the affected patients (aff) was given in Table 2. It was compared to the healthy controls (unaff). Odds ratios and p-values were calculated for each of the SNPs indifferent models (dominant, recessive).

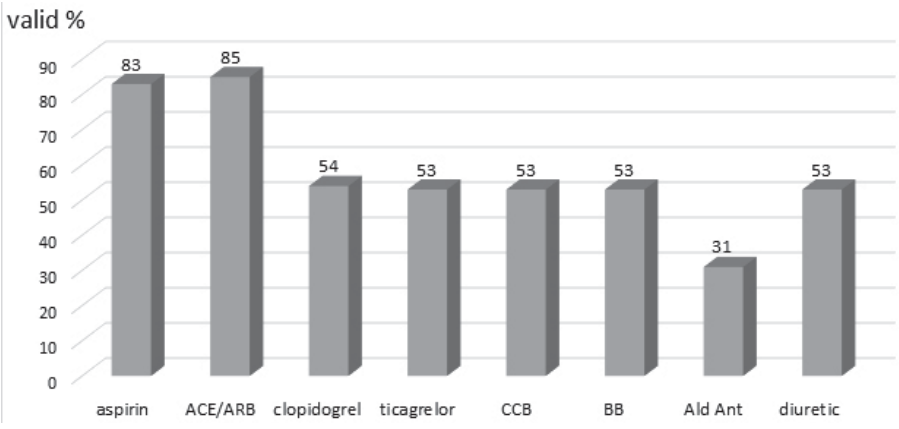


Fig. 1. Distribution of patients taking certain medication. CCB – calcium channel blockers, BB – beta-blockers, Ald Ant – aldosterone antagonists

Table 2. Comparison of the allelic and genotypic frequencies of the studied polymorphisms in the group of affected patients and unaffected population controls

CHR	SNP	Allele1	Allele 2	Model	AFFected patients	UNAFFected population controls	χ^2	DF	P value	OR	OR CI
10	rs11191548	C	T	GENO	1/23/83	6/44/142	NA	NA	0.56		
10	rs11191548	C	T	TREND	25/189	56/328	0.95	1	0.33		
10	rs11191548	C	T	ALLELIC	25/189	56/328	0.99	1	0.32	1.29	(0.78-2.14)
10	rs11191548	C	T	DOM	24/83	50/142	NA	NA	0.49	1.21	(0.69-2.12)
10	rs11191548	C	T	REC	1/106	6/186	NA	NA	0.28	3.41	(0.41-28.79)
11	rs381815	T	C	GENO	9/54/47	16/71/102	3.98	2	0.14		
11	rs381815	T	C	TREND	72/148	103/275	2.04	1	0.15		
11	rs381815	T	C	ALLELIC	72/148	103/275	2.02	1	0.15	0.77	(0.54-1.1)
11	rs381815	T	C	DOM	63/47	87/102	3.514	1	0.06	0.64	(0.40-1.02)
11	rs381815	T	C	REC	9/101	16/173	0.01	1	0.93	1.04	(0.44-2.43)

Note. Number of the chromosome (CHR); SNPs (SNP), alleles (A1, A2), test model, distribution of frequencies in the CAD patients (AFF = affected) and in the healthy controls (UNAFF = unaffected), level of significance (p – value), risk and confidence interval for the studied polymorphisms are presented in Table 2.

The T allele of rs11191548 showed increased risk effect for the development of CAD – OR above 1. In the recessive model, the OR was 3.41, but did not reach the accepted level of significance, and a larger sample might be needed to test for such.

We found a tendency for association with CAD when we compared patients vs population controls only for the dominant model of rs381815 (*PLEKHA7*) – CT+TT vs CC (P = 0.06, OR = 0,64, CI 0,4-1,02). The OR pointed towards protective effect of the variant. However, the result could not reach statistical significance (p = 0.06) (Figure 2).

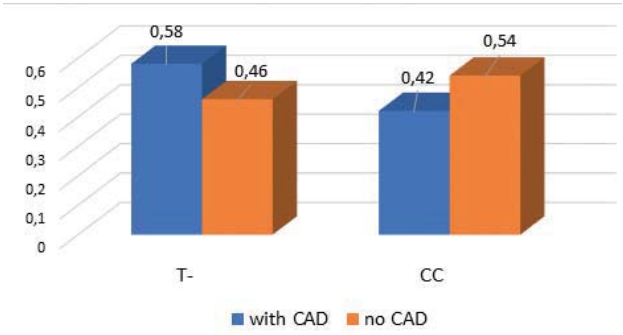


Figure 2. Distribution of the genotype frequencies for *PLEKHA7* rs381815 in dominant model

DISCUSSION

The aim of this study was to analyze the association of polymorphic variants ***rs11191548*** next to the ***CYP17A1*** gene and ***rs381815*** in the ***PLEKH7*** gene with CAD risk. It was done with "case-control" association analysis in a group of patients with angiographically documented CAD and healthy population controls. The genotyping was done with TaqMan® method as a quantitative analysis for genotyping of polymorphic variants.

Several polymorphisms, different form rs11191548 were studied for a potential association with CAD [19]. One previous association study found 13 new loci for CAD, including *CYP17A1* [20]. The International Consortium for Blood Pressure Genome-Wide Association Studies found 16 novel loci, some of which associated with CAD [21]. A genetic risk score based on 29 genome-wide significant variants was associated with AH, left ventricular hypertrophy, stroke, CAD.

A study of Dai et al. [22] in the Uygur population proved higher frequency of the C allele of rs4919686 in patients with CAD in comparison with healthy controls. For rs1004467 the C allele had protective role. For rs4919687 the frequency of the A allele was higher in patients with CAD. Association analysis of 13 loci, including rs12413409 of *CYP17A1* (which is usually associated with AH), have shown correlation with CAD [23, 24]. In a study of IBC 50K CAD Consortium, [25] rs3824755 polymorphism was associated with an elevated risk for CAD.

Butterworth et al. [26] selected 15596 patients with CAD and 34992 controls, and studied 2100 genes for 49094 genetic variants. They concluded that polymorphic variants in *CYP17A1* predispose to the development of CAD.

In 2015 year, Chuan-Fang Dai et al. studied 997 Chinese Han patients: 490 with CAD and 507 – controls. They assessed the role of rs4919686, rs1004467, rs4919687, rs10786712 and rs2486758 in *CYP17A1* in

relation to CAD. The polymorphic variant rs10786712 in recessive model was associated with CAD in men (P = 0.016). After adjustment for compromising factors, the difference was still significant (OR = 1.644, 95% [CI]: 1.087-2.488, P = 0.019). In women rs1004467 was associated with CAD in dominant model (P = 0.038). After adjustment for confounding factors the difference was still significant (OR = 1.623, 95% CI: 1.023-2.576, P = 0.040). The genotype distribution of rs4919687 showed significant difference between patients with CAD and controls in recessive model (P = 0.019), after correction OR = 0.417, 95% CI: 0.188-0.926, P = 0.032. The authors concluded that the polymorphisms rs1004467, rs4919687 and rs10786712 in *CYP17A1*were associated with the clinical manifestation of CAD in Chinese patients. The genotype TT of rs10786712 might have protective role in men, while the genotype CC of rs1004467 might have similar effect in women. Genotype AA of rs4919687 could bring excessive risk in women [27].

The polymorphism rs11191548 (minor C-allele) was associated with lower risk for hypertension and higher serum 25(OH) D levels in Han Chinese [28]. In a study of Chinese children Wu et al. [29], found association between rs11191548 near *CYP17A1* and HDL and leptin.

These studies pointed towards potential role (direct or indirect via HDL, leptin, sex hormones) of some polymorphic variants of *CYP17A1* in the pathogenesis of CAD.

In our study the frequency of allele T of the polymorphism rs11191548 was higher in patients with CAD but the difference did not reach statistical significance (p = 0.28, OR 3,41, CI 0.41-28.79) possibly because of the relatively small sample. In polygenic diseases, like CAD, the bigger the sample is, the clearer the association of the selected SNPs with the studied pathogenic condition will be. We tried to compensate for the limited sample size with careful and strict inclusion criteria – only patients with angiographically documented CAD with family history for early atherosclerosis.

The protein *PLEKHA7* participates in a complex with PDZD11. *PLEKHA7* is important for the stabilization of the adherent junctions and cytosolic microtubules. It ensures their integrity and thus is involved in the transmembrane Ca^{2+} flux [30, 31], and in arterial stiffness – very important preceding factor for arterial hypertension. The association between various polymorphisms in *PLEKHA7* gene and the development of AH was documented in many GWASs. When we discuss the potential role of *PLEKHA7* in the genesis of CAD, we should consider several aspects. First of all, its significance for the development of AH [32, 33, 34] and secondary to it – to CAD. Next, the role of *PLEKHA7* in the regulation of Ca^{2+} homeostasis and vasospasm [35] by NO-dependent route [36]. *PLEKHA7* elevates the NOS signaling in vascular smooth muscle cells [37], and is involved in the regulation of cell migration, apoptosis, and secondary plaque destabilization.

In conclusion, there are several mechanisms by which various polymorphisms in *PLEKHA7* can play a potential role in the pathogenesis of CAD of both the epicardial arteries and the microvasculature. Despite the theoretical considerations, the association between *PLEKHA7* and CAD has not been studied up to the present time.

In our study we found a tendency for association between the polymorphism rs381815 in *PLEKHA7* and CAD in the dominant model (CT+TT against CC) (p = 0.06; OR 0.64; CI 0.40-1.02). The OR pointed towards protective effect of the variant. The result did not reach statistical significance. In all other models of testing – genotypic, allelic, recessive, such a statistically significant difference between CAD patients and controls was not found as well. Thus, we cannot compare the results with the results from other studies. The association of rs381815 with blood pressure traits was documented in several studies [38]. Other polymorphisms in *PLEKHA7* were also associated with blood pressure variables in a targeted sequencing study of Morrison et al. [39] as well as in several GWASs [40, 41]. All of them tried to enhance the understanding of blood pressure regulation and explain the difference in medication response. However, any potential association between rs381815 and CAD was not studied.

Despite the inconclusive result, future studies of the association between *PLEKHA7* and CAD would be particularly important to further elucidate this association.

The earlier clinical manifestation in men in our results corresponded to the general findings for the sex difference in CAD [42]. We should emphasize the fact, that the number of women included in this study was lower, which is a problem of many CAD trials [4]. On the other hand, the earlier manifestation of CAD seen in men than in women with family history should be a starting point for future screening and prophylaxis in the Bulgarian population (Eastern European).

Most of our patients were with multiple cardio-vascular risk factors. It is known from different studies, that 72% of patients with AH have CAD [44, 45], and that in patients with diabetes [46] the morbidity of CAD is much higher than for the general population [47]. The isolated research of patients only with CAD without

any concomitant cardio-vascular risk factor is impossible in the everyday clinical practice, especially in a high-risk population like the Bulgarian one [48]. That, once again, underlines the necessity of early screening for CAD and cardiovascular risk factors. On the other hand, the precise genetic profile of a patient with CAD may aid their treatment, thus the patients with elevated genetic risk would benefit from therapy the most [49].

CONCLUSION

This was a study for the possible association between polymorphisms related to the development of AH and multiple metabolic pathways of sex hormones, corticosteroids (*CYP17A1*), Ca^{2+} exchange and intercellular connections (*PLEKHA7*). Two major principles could possibly theoretically correlate these genes to CAD: 1. directly – through a variety of mechanisms involved in Ca2+ exchange, intercellular communication, steroid hormones; 2. In directly – through AH and the variables of blood pressure. We studied the association of polymorphisms rs11191548 in *CYP17A1* gene and rs381815 in *PLEKHA7* gene and CAD.

When we tested the polymorphisms rs11191548 in *CYP17A1* gene and rs381815 in *PLEKHA7* and angiographically documented CAD with family history and/or early clinical manifestation, we did not find any significant association. For the polymorphism rs381815 in *PLEKHA7* we found a tendency for association with CAD in dominant model (TT+TC against CC) (p = 0.061; OR 0.64; CI 0.40-1.02) in comparison with healthy population controls. The variant rs11191548 in *CYP17A1* showed risk effect for the development of CAD – OR above 1 but the difference did not reach statistical significance. We cannot conclude based on the current results that there is association between the development of CAD and rs1191548 in *CYP17A1* or rs381815 in *PLEKHA7*. However, the result is important for driving future association studies in larger cohorts of Bulgarian CAD patients and healthy controls.

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