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Genetic Variants rs1049255 CYBA and rs2333227 MPO are Associated with Susceptibility to Coronary Artery Disease in Russian Residents of Central Russia

Aim	To study association of single-nucleotide polymorphisms rs1049255 CYBA and rs2333227 MPO with development of ischemic heart disease (IHD) in Russian residents of Central Russia.
Material and methods	The study material was DNA samples from 436 patients with IHD (265 men, 171 women; mean age, 61 years) and 370 sex- and age-matched arbitrarily healthy volunteers (209 men, 161 women; mean age, 60 years). Genotyping was performed by allelic discrimination with TaqMan probes.
Results	Comparative analysis of genotype frequency (log-additive regression model) showed that SNP rs1049255 CYBA (odds ratio, OR, 0.79 at 95% confidence interval, CI, from 0.65 to 0.96; p=0.02) and rs2333227 MPO (OR 0.72 at 95% CI from 0.55 to 0.95; p=0.02) were associated with a decreased risk of IHD adjusted for sex and age. Analysis of sex-specific effects showed that the protective effect of rs1049255 CYBA was evident only in men (OR 0.72 at 95% CI from 0.55 to 0.94; p=0.16).
Conclusion	The study demonstrated a protective effect of rs1049255 CYBA and rs2333227 MPO with respect of IHD in Russians. The protective effect of rs1049255 CYBA was observed only in men.
Keywords	Ischemic heart disease; oxidative stress; reactive oxygen species; rs1049255; CYBA; rs2333227; MPO; SNP; sexual dimorphism
For citation	Bushueva O. Yu. Genetic Variants rs1049255 CYBA and rs2333227 MPO are Associated with Susceptibility to Coronary Artery Disease in Russian Residents of Central Russia. Kardiologiia. 2020;60(10):47–54. [Russian: Бушуева О. Ю. Генетические варианты rs1049255 CYBA и rs2333227 MPO ассоциированы с предрасположенностью к ишемической болезни сердца русских жителей Центральной России. Кардиология. 2020;60(10):47–54]
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Coronary artery disease (CAD) remains a major pathology in the context of global morbidity and mortality [1]. The data accumulated over the past decades provide major evidence of the role of oxidative stress in the onset and progression of CAD [2, 3]. Oxidative stress is defined as an imbalance between the generation of reactive oxygen species (ROS) and the activity of antioxidants. Oxidative stress results in the formation of oxidized low-density lipoprotein (LDL), which significantly contributes to atherogenesis through several mechanisms. These include activation and dysfunction of endothelial cells, formation of macrophage foam cells, and migration and proliferation of smooth muscle cells [4]. Moreover, a direct correlation between the NADPH-oxidase-generated ROS and levels of oxidized LDL and the stability of atherosclerotic plaques [5] was found, which confirms a direct association with the development of coronary atherosclerosis and CAD. Variations in the genes encoding the redox-homeostasis enzymes can influence the functional activity of the respective enzymes and, thus, determine susceptibility to oxidative stress.

NADPH-oxidase is necessary for oxidase activity in smooth muscle cells and is the main source of superoxide in the vasculature [6]. Single nucleotide polymorphisms (SNP) of A640G (rs1049255) in CYBA 3'UTR, which encodes the p22phox subunit of NADPH-oxidase, are localized within the miRNA binding site and can influence CYBA expression and NADPH-oxidase activity [7]. Studies of the association of single nucleotide polymorphisms in the CYBA's A640G polymorphic site with the risk of developing CAD have produced contradictory findings. For example, Macias–Reyes et al. [8] found an association of the G/G genotype of rs1049255 in CYBA with CAD in the Spanish population; however, this SNP was not associated with the development of CAD in the Chinese Han population [9].

Myeloperoxidase (MPO) is another enzyme with pro-oxidant activity. MPO was first discovered in phagocytes and is an integral component of antimicrobial protection [10]. During an oxidative burst, MPO generates significant amounts of ROS, such as hypochloric acid, tyrosyl radicals, and chemically reactive nitrogen particles. These metabolites were shown to be involved in the processes of oxidative damage in cardiovascular disease (CVD) [11]. Polymorphism -463G>A in the promoter region of MPO (rs233227) leads to the loss of the binding site of the transcription factor SP1 and significantly affects gene expression [12].

Increased MPO concentrations were adequately demonstrated to be independently associated with an increased risk of CAD [13]. However, there were few studies of the association of SNP rs233227 (-463G>a) in MPO with the development of CAD. For example, a Canadian study [14] in 2001 was the first to show the protective effect of polymorphism -463G>a with respect to the risk of CAD. Based on the studies of mainly Chinese populations, the meta-analysis performed by Tang et al. [15] in 2013 found that the G/A and A/A genotypes were significantly associated with decreased risk of CAD [15].

However, another meta-analysis, which included five case-control studies performed in the Dutch, Canadian, Swedish, Turkish and Chinese populations, showed no association between this polymorphism and the risk of developing CAD [16]. Mandsorwale et al. [17] found no association of the polymorphism -463G>A with CAD in the Indian population. Inconsistency of these findings may be due to genetic differences of the studied populations. There have been no studies on the relationship of gene polymorphisms with the pro-oxidant effect of CYBA rs1049255 and MPO rs233227 with CAD in the Russian population.

Objective

The objective was to study the association of single nucleotide polymorphisms CYBA rs1049255 and MPO rs233227 with the development of CAD in residents of Central Russia.

Material and Methods

The study material was a sample of unrelated Russian individuals living in the Kursk region for at least three generations (n=806). The study included 436 patients with CAD (265 males, 171 females) who were hospitalized at the Kursk Regional Clinical Hospital and at the Kursk City Clinical Hospital for Emergency Care between 2011 and 2017 [18]. The comparison group comprised 370 apparently healthy volunteers (209 males, 161 females) without a history of chronic disease, who had normal blood pressure (BP) and did not receive antihypertensive therapy [19]. The control sample included healthy volunteers, personnel undergoing medical check-ups at industrial enterprises, and healthcare providers in Kursk and the Kursk region. Before the collection of biological material, BP was measured twice, with an interval of 5 min. The samples of patients and healthy volunteers were continuous. The sex of the CAD and control groups were comparable (p=0.41, Table 1). The mean age of patients with CAD was 61 yrs; the mean age of the control subjects was 60 yrs (p=0.57).

The inclusion criteria were confirmed CAD diagnosed based on the medical history and examination, i.e., presence of ischemic changes in stress electrocardiogram, echocardiographic visualization of hypo- and akinesia areas at rest or stress, detection of clinically significant atherosclerotic lesions using coronary angiography. Patients with chronic liver, kidney diseases (creatinine clearance < 60 ml/min/1.73 m^2), cancer, cardiomyopathy, chronic obstructive pulmonary disease, diffuse connective tissue diseases, acute and chronic inflammatory infections, endocrine diseases (including type 2 diabetes mellitus), anemia, or alcoholism were excluded from the study. Patients with a history of cerebral stroke were also excluded. All patients with CAD had arterial hypertension (stage III, risk 4) and chronic heart failure.

The study was conducted according to the Declaration of Helsinki. The regional ethics committee of Kursk State Medical University approved the study protocol. All patients signed an informed consent form to be included in the study.

Venous blood samples were taken from all subjects. Genomic DNA was isolated by the standard phenol-chloroform extraction procedure. CYBA rs1049255 and MPO rs233227 were genotyped by real-time polymerase chain reaction using published genotyping techniques on a Bio-Rad Cfx96 amplifier [20]. Sintol oligonucleotide primers and probes were used. Repeated genotyping of 10% of the studied samples, selected randomly and blinded to the disease status, showed 100% reproducibility of the initial results.

Statistical analysis of the obtained data was carried out with R software. Normality was tested with the Shapiro-Wilk and der Spiegelhalter tests. Levene's test was used to check the homogeneity of group dispersions in the normal distribution of signs. Since the data distribution was non-normal in most cases and/or the dispersion equality condition was not observed, the non-parametric Mann – Whitney rank sum test used to compare quantitative data. The level of statistical significance for the comparison of nominal data was calculated using the Yates' χ^2 test. To eliminate the effect of multiple comparisons, the Benjamin-Hochberg procedure was used to correct the resulting p-values. The average levels of the sample data were described using the median and the 1st and 3rd quartiles (Me [Q1; Q3]). Logistic regression analysis was used to analyze associations between alleles and the risk of CAD. The association between genotypes and the disease was estimated using the odds ratio (OR) and 95% confidence interval (CI) as calculated for the sex- and age-specific log-additive regression model in the SNPStats web tool (https://www.snpstats.net/start.htm). Differences were considered statistically significant at p≤0.05. To assess

ISSN 0022-9040. Kardiologiia. 2020;60(10). DOI: 10.18087/cardio.2020.10.n1229

the regulatory potential of the polymorphic gene variants, bioinformation resources were used: HaploReg (v4.1) (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php), QTLbase (http://mulinlab.org/qtlbase), and GTex portal (https://gtexportal.org/home/).

Results

The clinical characteristics of the study subjects are provided in Table 1. In the general sample, the frequency distributions of the CYBA rs1049255 and MPO rs233227 genotypes corresponded to the Hardy-Weinberg equilibrium (p>0.05). However, in the control group, CYBA rs1049255 deviated from the Hardy-Weinberg equilibrium due to an increase in observed heterozygosity (p>0.05).

Table 1. Clinical characteristics of the study groups

Parameter		Patients with CAD (n=436)	Control (n=370)	p
Age, yrs, Me [Q1; Q3]		61 [55; 69]	60 [56; 68]	0.57
Sex	Male, n (%)	265 (60.8)	209 (56.5)	0.41
	Female, n (%)	171 (39.2)	161 (43.5)	
Smoking	Smokers, n (%)	157 (44)	139 (40.8)	0.542
	Non-smokers, n (%)	200 (56)	202 (59.2)	
	Smoking males	143 (91.1)	128 (92.1)	0.76
	Smoking females	14 (8.9)	11 (7.9)	
	Non-smoking males	68 (34)	67 (33.2)	0.86
	Non-smoking females	132 (66)	135 (66.8)	
Body mass index, Me [Q1; Q3]		24.3 [22.5; 27.1]	23.9 [22.7; 26.4]	0.64
Nature of the main activity, n (%)	physical	111 (47.6)	57 (60)	0.02
	intellectual	122 (52.4)	38 (40)	
Family history of CVDs	yes, n (%)	138 (79.3)	44 (40)	2.05×10^{-10}
	no, n (%)	36 (20.7)	66 (60)	
Cholesterol, mmol/l, Me [Q1; Q3]		5.4 [4.58; 6.02]	–	–
Triglycerides, mmol/l, Me [Q1; Q3]		1.37 [0.99; 2]	–	–
Age of AH manifestation, yrs, Me [Q1; Q3]		50 [44; 55]	–	
Age of CAD manifestation, yrs, Me [Q1; Q3]		52 [47; 57]	–	–
Angina functional class	II, n (%)	56 (37.1)	–	–
	III, n (%)	90 (59.6)	–	–
	IV, n (%)	5 (3.3)	–	–
CHF functional class	I, n (%)	33 (17.6)	–	–
	II, n (%)	152 (81.3)	–	–
	III, n (%)	2 (1.1)	–	–
History of MI	yes, n (%)	115 (26.4)	–	–
	no, n (%)	321 (73.6)	–	–

Data are Me [Q1; Q3] or n (%). CAD, coronary artery disease; CVD, cardiovascular disease; CHF, chronic heart failure; AH, arterial hypertension; MI, myocardial infarction.

Table 2 provides models of genotype associations of the studied SNP with the development of CAD. A log-additive regression model was used to interpret the results of the association analysis since it involves the analysis of the additive model of transmission and it allows estimating the contribution of each allele independently of the contribution of other alleles. The protective effects of G allele of CYBA rs1049255 (OR 0.80, 95% CI 0.66–0.97; p=0.03) and A allele of MPO rs233227 (OR 0.72, 95% CI 0.55–0.94; p=0.02) were identified with respect to the risk of developing CAD (Table 2). The comparative analysis of the genotype frequencies also showed that SNPs CYBA rs1049255 (OR 0.79, 95% CI 0.65–0.96; p=0.02) and MPO rs233227 (OR 0.72, 95% CI 0.55–0.95; p=0.018) were associated with reduced risk of CAD (Table 2).

Given the sexual dimorphism in the manifestation of genetic marker associations that are characteristic of multifactorial diseases, a sex-specific differentiated analysis was conducted (Table 3). Males had a protective effect of the G allele of CYBA rs1049255 with respect to the risk of developing CAD at borderline statistical significance level (OR 0.77, 95% CI 0.60–1.00; p=0.049). The log-additive model of genotype associations identified a protective effect of CYBA rs1049255 with respect to the risk of developing CAD in males (OR 0.72, 95% CI 0.55–0.94; p=0.016). No associations were found in women.

Discussion

NADPH-oxidases producing superoxide anionic radical as a result of NADPH or NADH oxidation are the dominant source of superoxide anion radicals in coronary arteries [5]. NADPH-oxidases are most expressed in fibroblasts, endothelial, smooth muscle, and in immune cells that infiltrate the vascular walls, and in phagocytes [21]. NADPH-oxidase's molecular complex consists of membrane-associated subunits, one of which is p22phox, so-called cytochrome b, or CYBA. Since the CYBA subunit is a channel for NADPH-oxidase electron transport through the membrane, it most affects NADPH-oxidase activity. Polymorphism 640A>G, which is localized in the 3′-non-translated region of the gene, does not cause amino acid replacement. However, the AA genotype was shown to be associated with a 30% increase in ROS production compared to that of GG homozygotes [22]. Interestingly, rs1049255 cis-eQTL associated with the risk alleles A (T) in CYBA is associated with the increased gene expression in the aorta (normalized effect size NES=0.05; p=0.029), coronary arteries (NES=0.01; p=0.766), whole blood

Table 2. Analysis of associations of the single nucleotide polymorphisms CYBA rs1049255 and MPO rs233227 with the risk of developing CAD

Association models		Minor allele/ Genotypes	Control (n=370)	Patients with CAD (n=436)	OR (95% CI)	p*
Genetic models of the associations of CYBA rs1049255 with the development of CAD						
Allele associations		G	0.51	0.46	**0.80 (0.66–0.97)**	**0.03**
Genotype associations	Codominant model	A/A	74 (20%)	136 (31.2%)	1	**0.0005**
		A/G	211 (57%)	200 (45.9%)	**0.51 (0.36–0.72)**	
		G/G	85 (23%)	100 (22.9%)	**0.63 (0.42–0.95)**	
	Dominant model	A/A	74 (20%)	136 (31.2%)	**1**	**0.0002**
		A/G-G/G	296 (80%)	300 (68.8%)	**0.54 (0.39–0.75)**	
	Recessive model	A/A-A/G	285 (77%)	336 (77.1%)	1	0.97
		G/G	85 (23%)	100 (22.9%)	0.99 (0.71–1.38)	
	Superdominant model	A/A-G/G	159 (43%)	236 (54.1%)	1	**0.0013**
		A/G	211 (57%)	200 (45.9%)	**0.63 (0.48–0.84)**	
	Log-additive model	–	–	–	**0.79 (0.65–0.96)**	**0.02**
Genetic models of the rs2333227 MPO associations with the development of CAD						
Allele associations		A	0.184	0.140	**0.72 (0.55–0.94)**	0.02
AGenotype associations	Codominant model	G/G	246 (66.5%)	320 (73.4%)	1	0.045
		G/A	112 (30.3%)	110 (25.2%)	0.76 (0.56–1.04)	
		A/A	12 (3.2%)	6 (1.4%)	0.38 (0.14–1.03)	
	Dominant model	G/G	246 (66.5%)	320 (73.4%)	1	0.038
		G/A-A/A	124 (33.5%)	116 (26.6%)	**0.73 (0.54–0.98)**	
	Recessive model	G/G-G/A	358 (96.8%)	430 (98.6%)	1	0.068
		A/A	12 (3.2%)	6 (1.4%)	0.41 (0.15–1.10)	
	Superdominant model	G/G-A/A	258 (69.7%)	326 (74.8%)	1	0.13
		G/A	112 (30.3%)	110 (25.2%)	0.79 (0.58–1.07)	**0.018**
	Log-additive model	–	–	–	**0.72 (0.55–0.95)**	

*, p with sex and age correction; CAD, coronary artery disease; OR, odds ratio; CI, confidence interval.

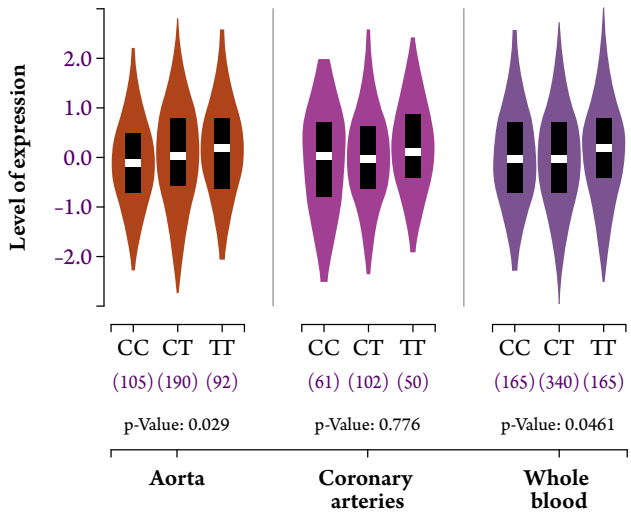
Table 3. Analysis of the associations of the single nucleotide polymorphisms CYBA rs1049255 and MPO rs233227 with the sex-specific risk of CAD

Gene/SNP	Minor allele/ Genotypes	Control	Patients with CAD	OR (95% CI)	p*
Male		**n=209**	**n=265**		
rs1049255 CYBA	G	0.519	0.455	**0.77 (0.60–1.00)**	**0.049**
	A/A	38 (18.2%)	84 (31.7%)	**0.72 (0.55–0.94)****	**0.016****
	A/G	125 (59.8%)	121 (45.7%)		
	G/G	46 (22%)	60 (22.6%)		
rs2333227 MPO	A	0.179	0.142	0.75 (0.53–1.07)	0.13
	G/G	142 (67.9%)	192 (72.5%)	0.75 (0.52–1.07)**	0.12**
	G/A	59 (28.2%)	71 (26.8%)		
	A/A	8 (3.8%)	2 (0.8%)		
Female		**n=161**	**n=171**		
rs1049255 CYBA	G	0.509	0.456	0.84 (0.62–1.14)	0.3
	A/A	36 (22.4%)	52 (30.4%)	0.82 (0.60–1.11)**	0.2**
	A/G	86 (53.4%)	79 (46.2%)		
	G/G	39 (24.2%)	40 (23.4%)		
rs2333227 MPO	A	0.189	0.137	0.68 (0.45–1.03)	0.08
	G/G	104 (64.6%)	128 (74.8%)	0.66 (0.43–1.01)**	0.052**
	G/A	53 (32.9%)	39 (22.8%)		
	A/A	4 (2.5%)	4 (2.3%)		

*, p with age correction; **, values calculated for log-additive regression model with sex and age correction; CAD, coronary artery disease; OR, odds ratio; CI, confidence interval.

ISSN 0022-9040. Kardiologiia. 2020;60(10). DOI: 10.18087/cardio.2020.10.n1229

Figures 1. Effects of carriership of various rs1049255 genotypes on CYBA expression in aorta, coronary arteries and whole blood, according to GTEx Portal



(NES=0.03; p=0.046), and therefore with increased generation of ROS in these target tissues (https://gtexportal.org/; Figure 1).

According to HaplReg (v4.1), CYBA rs1049255 is located in the DNA binding region with histone H3, which is characterized by lysine 4 (H3K4me1) monomethylation and marks the enhancers in proinflammatory monocytes CD14+. The effect of this histone mark is enhanced by the acetylation of lysine-27 H3 (H3K27ac) histone, which marks the enhancers in primary peripheral blood monocytes, the right ventricle, and CD14+ monocytes. Subsequent bioinformatic analysis of methylation quantitative trait loci (mQTL) revealed that the risk allele A of CYBA rs1049255 was bound to cis-mQTL associated with decreased DNA methylation in peripheral blood (Table 4). Hence, the transport action of this allele can be associated with the increased CYBA expression through the mechanisms of mQTL-associated methylation reduction. Thus, according to bioinformatic analysis, CYBA rs1049255 is characterized by high regulatory potential, including through the epigenetic mechanisms of gene expression regulation.

The study also found that CYBA rs1049255 is associated with CAD development in males only and is not associated with the predisposition for this disease in females. These differences may be based on two main mechanisms. First, BP regulation and the predisposition for atherosclerosis are sex-specific. This is due not only to differential regulation of nitric oxide (NO) bioavailability, but also to sex-related differences in the generation of superoxide anionic radical [23] that is mainly formed by NADPH-oxidases, e.g., CYBA. Second, there were far more smokers among males than females. Given that cigarette smoke is the main source of superoxide [24], excess superoxide may be expected to contribute to an increased risk of developing CAD in males due to the effects of cigarette smoke components combined with the carrier action of the functionally more active A allele of CYBA rs1049255.

MPO rs233227 was another SNP associated with the development of CAD in this Central Russian population. MPO -463G>a polymorphism can be associated with susceptibility to CAD through several molecular mechanisms. MPO was shown to promote endothelial dysfunction and vasoconstriction by increasing NO consumption [25]. MPO can consume NO in a catalytic reaction, acting as its catalytic absorber, and thus limiting NO in vivo bioavailability. Moreover, the MPO-generated hypochlorous acid can chemically react directly with L-arginine, a key substrate for nitric oxide synthase (NOS). The resulting chlorinated L-arginine inhibits NO production in endothelial cells and decreases endothelial vasorelaxation [26]. By its oxidative modification and remodeling and due to its mediated activation of metalloproteinases, MPO can also contribute to the development of CVD by increasing the stiffness of the arterial wall [27].

Analysis of quantitative trait loci showed that MPO rs23323227 is bound to cis-mQTL associated with the increased methylation of four CpG sites in MPO (see Table 5). Thus, increased MPO methylation in the A allele carriers can influence the decrease in its expression and result in reduced production of ROS. This can also determine the protective mechanisms of the A allele with respect to the risk of developing CAD. Interestingly, the A allele of rs23323227 MPO is bound to cis-pQTL that reduce the expression of the MPO protein (P05164) in peripheral blood (p=1.17×10–10), especially in patients with CVD of atherosclerotic origin [28].

According to HaploReg (v4.1), MPO rs233227 is located in the DNA binding region with modified histone H3K4me1, which marks the enhancers (in peripheral blood monocytes, CD14+ monocytes), with modified histone H3K4me^3, which marks promoters (in the right ventricle, monocytes CD14+), and with modified histone H3K27ac, which marks the enhancers (in the primary peripheral blood monocytes, the right ventricle, CD14+ monocytes). Thus, the detected regulatory effects of MPO rs233227 are implemented through a wide range of epigenetic mechanisms.

Conclusion

The association of CYBA rs1049255 and MPO rs233227 with the development of CAD has been established for the first time in the Central Russian population. Given the pronounced pro-oxidant effects of these genes and the

Table 4. Cis-mQTL associated with the risk allele A of CYBA rs1049255 in peripheral blood

CpG-site (genomic region)	Effect size (beta)	p[1]	FDR[2]
cg05463589 (chr16:88706426)	–0.35405	4.83×10^{-18}	2.33×10^{-12}
cg05463589 (chr16:88706426)	–0.36935	9.38×10^{-18}	4.34×10^{-12}
cg00362322 (chr16:88704230)	–0.0273	1.29×10^{-16}	–
cg21593409 (chr16:88706389)	–0.34401	2.28×10^{-16}	9.74×10^{-11}
cg05463589 (chr16:88706426)	–0.36793	2.68×10^{-16}	1.21×10^{-10}
cg05463589 (chr16:88706426)	–0.35159	1.59×10^{-15}	8.55×10^{-10}
cg00362322 (chr16:88704230)	–0.32524	3.57×10^{-15}	1.35×10^{-9}
cg00362322 (chr16:88704230)	–0.32951	7.85×10^{-15}	2.88×10^{-9}
cg21593409 (chr16:88706389)	–0.30911	2.78×10^{-14}	9.7×10^{-9}
cg05463589 (chr16:88706426)	–0.32285	3.1×10^{-13}	1.13×10^{-7}
cg05202654 (chr16:88706241)	–0.2978	7.1×10^{-12}	1.91×10^{-6}
cg21593409 (chr16:88706389)	–0.29932	2.91×10^{-11}	7.93×10^{-6}
cg05202654 (chr16:88706241)	–0.29788	4.61×10^{-11}	1.22×10^{-5}
cg00362322 (chr16:88704230)	–0.28602	8.3×10^{-10}	0.000199
cg08155347 (chr16:88704957)	–0.2629	1.45×10^{-9}	0.000273
cg00816037 (chr16:88812342)	–0.245	1.63×10^{-9}	0.000306
cg05202654 (chr16:88706241)	–0.25173	9.04×10^{-9}	0.00152
cg26748794 (chr16:88804051)	–0.24464	1.71×10^{-8}	0.00275
cg21593409 (chr16:88706389)	–0.24545	3.17×10^{-8}	0.00583
cg00362322 (chr16:88704230)	–0.25588	4.21×10^{-8}	0.00719
cg26748794 (chr16:88804051)	–0.23896	6.85×10^{-8}	0.01

Table 4 includes the results of European population studies provided QTLbase (http://mulinlab.org/qtlbase)[1], p-level of significance; [2], p-level of significance corrected for false discovery rate (FDR).

Table 5. Cis-mQTL associated with the protective allele A rs2333227 MPO in peripheral blood

#	CpG-site (genomic region)	Effect size (beta)	p[1]	FDR[2]
1.	cg13734043 (chr17:56270198)	0,283395	2×10^{-8}	0,01
2.	cg13734043 (chr17:56270198)	0,26839	$1,2\times10^{-8}$	0,005
3.	cg13734043 (chr17:56270198)	0,31877	$1,49\times10^{-9}$	0,001
4.	cg13734043 (chr17:56270198)	0,281783	$9,47\times10^{-10}$	0,001

Table 5 includes the results of European population studies provided QTLbase (http://mulinlab.org/qtlbase); p[1], level of significance; p FDR[2], level of significance corrected for false discovery rate (FDR).

proven, significant role of reactive oxygen species in the development of coronary atherosclerosis, the mechanisms of the established associations are most likely to be due to the protective effects of alleles linked with reduced oxidant production. Moreover, we have shown that the protective effect of G allele of CYBA rs1049255 appears only in men, which provides further evidence of the significant role of sex-specific effects in forming a predisposition to CAD. It should also be noted that the bioinformatic analysis of CYBA rs1049255 and MPO rs233227 revealed the high regulatory potential of the studied single nucleotide polymorphisms, which suggests a potentially important role of epigenetic mechanisms in the regulation of gene expression. These results can be used to develop new approaches for preventing and treating CAD based on combating oxidative stress.

ISSN 0022-9040. Kardiologiia. 2020;60(10). DOI: 10.18087/cardio.2020.10.n1229

Acknowledgements

The author thanks the Distinguished Scientist of the Russian Federation V.P. Ivanov, MD, Professor of the Biology, Medical Genetics and Ecology Department of the Kursk State Medical University and A.V. Polonikov, MD, Professor of the Biology, Medical Genetics and Ecology Department and Director of the Research Institute of Genetic and Molecular Epidemiology of the Kursk State Medical University, for their valuable consulting at key stages of this study.

Funding

The study was supported by the Kursk State Medical University.

No conflict of interest is reported.

The article was received on 25/06/2020

REFERENCES

1. Laslett LJ, Alagona P, Clark BA, Drozda JP, Saldivar F, Wilson SR et al. The Worldwide Environment of Cardiovascular Disease: Prevalence, Diagnosis, Therapy, and Policy Issues. Journal of the American College of Cardiology. 2012;60(25):S1–49. DOI: 10.1016/j.jacc.2012.11.002
2. Lubrano V, Pingitore A, Traghella I, Storti S, Parri S, Berti S et al. Emerging Biomarkers of Oxidative Stress in Acute and Stable Coronary Artery Disease: Levels and Determinants. Antioxidants. 2019;8(5):115. DOI: 10.3390/antiox8050115
3. Golubenko M.V., Babushkina N.P., Zarubin A.A., Salakhov R.R., Makeeva O.A., Markova V.V. et al. Association of the mitochondrial DNA haplogroup H1 variants with the risk of acute cardiovascular events. Research Results in Biomedicine. 2019;5(4):19–31. [Russian: Голубенко М.В., Бабушкина Н.П., Зарубин А.А., Салахов Р.Р., Макеева О.А., Маркова В.В. и др. Ассоциация вариантов гаплогруппы H1 митохондриальной ДНК с риском сердечно-сосудистых катастроф. Научные результаты биомедицинских исследований. 2019;5(4):19-31]. DOI: 10.18413/2658-6533-2019-5-4-0-2
4. Borén J, Chapman MJ, Krauss RM, Packard CJ, Bentzon JF, Binder CJ et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease: pathophysiological, genetic, and therapeutic insights: a consensus statement from the European Atherosclerosis Society Consensus Panel. European Heart Journal. 2020;41(24):2313–30. DOI: 10.1093/eurheartj/ehz962
5. Sorescu D, Weiss D, Lassègue B, Clempus RE, Szöcs K, Sorescu GP et al. Superoxide Production and Expression of Nox Family Proteins in Human Atherosclerosis. Circulation. 2002;105(12):1429–35. DOI: 10.1161/01.CIR.0000012917.74432.66
6. Prieto-Bermejo Rodrigo, Hernández-Hernández Angel. The Importance of NADPH Oxidases and Redox Signaling in Angiogenesis. Antioxidants. 2017;6(2):32. DOI: 10.3390/antiox6020032
7. Liu C, Rennie WA, Carmack CS, Kanoria S, Cheng J, Lu J et al. Effects of genetic variations on microRNA: target interactions. Nucleic Acids Research. 2014;42(15):9543–52. DOI: 10.1093/nar/gku675
8. Macías-Reyes A, Rodríguez-Esparragón F, Caballero-Hidalgo A, Hernández-Trujillo Y, Medina A, Rodríguez-Pérez JC. Insight into the role of CYBA A640G and C242T gene variants and coronary heart disease risk. A case-control study. Free Radical Research. 2008;42(1):82–92. DOI: 10.1080/10715760701796918
9. Liu S, Li X, Wang J, Ji J, Liu J, Lin Y et al. The rs9932581 and rs1049255 Polymorphisms in CYBA is not Associated with Preeclampsia in Chinese Han Women. Cellular Physiology and Biochemistry. 2016;39(4):1471–8. DOI: 10.1159/000447850
10. Winterbourn CC, Vissers MC, Kettle AJ. Myeloperoxidase: Current Opinion in Hematology. 2000;7(1):53–8. DOI: 10.1097/00062752-200001000-00010
11. Zelzer S, Enko D, Pilz S, Tomaschitz A, März W, Meinitzer A. Myeloperoxidase, asymmetric dimethyl-arginine and the renin-angiotensin-aldosterone-system in cardiovascular risk patients: Cross-sectional findings from the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. Clinical Biochemistry. 2017;50(13–14):739–45. DOI: 10.1016/j.clinbiochem.2017.03.013
12. Desikan R, Narasimhulu CA, Khan B, Rajagopalan S, Parthasarathy S. Myeloperoxidase (MPO): Do We Need Inhibitors? In: Mechanisms of Vascular Defects in Diabetes Mellitus [ISBN: 978-3-319-60323-0]. Kartha CC, Ramachandran S, Pillai RM, editors -Cham: Springer International Publishing; 2017. - P. 535-571. [DOI: 10.1007/978-3-319-60324-7_24].
13. Afshinnia F, Zeng L, Byun J, Gadegbeku CA, Magnone MC, Whatling C et al. Myeloperoxidase Levels and Its Product 3-Chlorotyrosine Predict Chronic Kidney Disease Severity and Associated Coronary Artery Disease. American Journal of Nephrology. 2017;46(1):73–81. DOI: 10.1159/000477766
14. Nikpoor B, Turecki G, Fournier C, Théroux P, Rouleau GA. A functional myeloperoxidase polymorphic variant is associated with coronary artery disease in French-Canadians. American Heart Journal. 2001;142(2):336–9. DOI: 10.1067/mhj.2001.116769
15. Tang N, Wang Y, Mei Q. Myeloperoxidase G-463A polymorphism and susceptibility to coronary artery disease: A meta-analysis. Gene. 2013;523(2):152–7. DOI: 10.1016/j.gene.2013.03.131
16. Chang C, Gao B, Liu Z, Mao J, Jiang G. The myeloperoxidase -463G/A polymorphism and coronary artery disease risk: A meta-analysis of 1938 cases and 1990 controls. Clinical Biochemistry. 2013;46(16–17):1644–8. DOI: 10.1016/j.clinbiochem.2013.09.002
17. Mandsorwale D, Nagtilak S, Lalchandani A, Srivastava AK. Role of-463 G/A genetic polymorphism & myeloperoxidase activity in prediction of cardiovascular disease. Scholars Journal of Applied Medical Sciences. 2014;2(4D):1402–7. [Av. at: http://saspublisher.com/wp-content/uploads/2014/07/SJAMS-24D1402-1407.pdf]
18. Polonikov A, Kharchenko A, Bykanova M, Sirotina S, Ponomarenko I, Bocharova A et al. Polymorphisms of CYP2C8 , CYP2C9 and CYP2C19 and risk of coronary heart disease in Russian population. Gene. 2017;627:451–9. DOI: 10.1016/j.gene.2017.07.004
19. Bushueva O, Solodilova M, Ivanov V, Polonikov A. Gender-specific protective effect of the -463G>A polymorphism of myeloperoxidase gene against the risk of essential hypertension in Russians. Journal of the American Society of Hypertension. 2015;9(11):902–6. DOI: 10.1016/j.jash.2015.08.006
20. Packer BR, Yeager M, Burdett L, Welch R, Beerman M, Qi L et al. SNP500Cancer: a public resource for sequence validation, assay development, and frequency analysis for genetic variation in candidate genes. Nucleic Acids Research. 2006;34(90001):D617–21. DOI: 10.1093/nar/gkj151
21. Katsuyama M. NOX/NADPH Oxidase, the Superoxide-Generating Enzyme: Its Transcriptional Regulation and Physiological Roles. Journal of Pharmacological Sciences. 2010;114(2):134–46. DOI: 10.1254/jphs.10R01CR
22. Schirmer M, Hoffmann M, Kaya E, Tzvetkov M, Brockmöller J. Genetic polymorphisms of NAD(P)H oxidase: variation in subunit expression and enzyme activity. The Pharmacogenomics Journal. 2008;8(4):297–304. DOI: 10.1038/sj.tpj.6500467
23. Dantas A, Franco M, Silva-Antonialli MM, Tostes RC, Fortes ZB, Nigro D et al. Gender differences in superoxide generation in microvessels of hypertensive rats: role of NAD(P)H-oxidase. Cardiovascular Research. 2004;61(1):22–9. DOI: 10.1016/j.cardiores.2003.10.010
24. Dikalov S, Itani H, Richmond B, Arslanbaeva L, Vergeade A, Rahman SMJ et al. Tobacco smoking induces cardiovascular mitochondrial oxidative stress, promotes endothelial dysfunction, and enhanc-

es hypertension. American Journal of Physiology-Heart and Circulatory Physiology. 2019;316(3):H639–46. DOI: 10.1152/ajpheart.00595.2018
25. Baldus S, Rudolph V, Roiss M, Ito WD, Rudolph TK, Eiserich JP et al. Heparins Increase Endothelial Nitric Oxide Bioavailability by Liberating Vessel-Immobilized Myeloperoxidase. Circulation. 2006;113(15):1871–8. DOI: 10.1161/CIRCULATIONAHA.105.590083
26. Yang J, Cheng Y, Ji R, Zhang C. Novel model of inflammatory neointima formation reveals a potential role of myeloperoxidase in neointimal hyperplasia. American Journal of Physiology-Heart and Circulatory Physiology. 2006;291(6):H3087–93. DOI: 10.1152/ajpheart.00412.2006
27. Rodríguez-Pla A, Bosch-Gil JA, Rosselló-Urgell J, Huguet-Redecilla P, Stone JH, Vilardell-Tarres M. Metalloproteinase-2 and -9 in Giant Cell Arteritis: Involvement in Vascular Remodeling. Circulation. 2005;112(2):264–9. DOI: 10.1161/CIRCULATIONAHA.104.520114
28. Yao C, Chen G, Song C, Keefe J, Mendelson M, Huan T et al. Genome-wide mapping of plasma protein QTLs identifies putatively causal genes and pathways for cardiovascular disease. Nature Communications. 2018;9(1):3268. DOI: 10.1038/s41467-018-05512-x

ISSN 0022-9040. Kardiologiia. 2020;60(10). DOI: 10.18087/cardio.2020.10.n1229