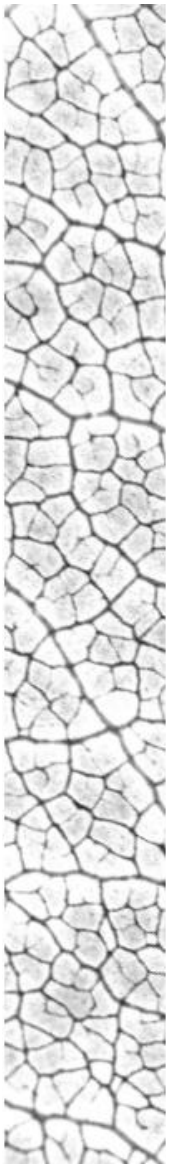

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The study of the associations of polymorphism of matrix metalloproteinases with multifactorial human diseases and in silico assessment of their functional effects

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Abstract
The objective of this study is to identify the associations of polymorphism of matrix metalloproteinase genes with multifactorial diseases and evaluate *in silico* their functional effects. The material for the study was genomic DNA isolated from venous blood samples by the standard phenol-chloroform extraction method. The rs2250889 MMP9, rs11568818 MMP7 and rs17576 MMP9 polymorphic loci were studied using the polymerase chain reaction on a CFX-96 Real-Time System thermal cycler (Bio-Rad Laboratories, Inc., US) using oligonucleotide primers and probes synthesized by Syntol LLC, Russia). It was established that the rs2250889 *MMP9* polymorphic locus is associated with the development of primary open-angle glaucoma (OR=0.67-1.44, p=0.02-0.04), the rs11568818 *MMP7* locus - with arterial hypertension (OR=0.69-0.84, p=0.03), the rs17576 *MMP9* locus - with peptic ulcer (OR=1.24, p=0.04). With the help of HaploReg (v4.1), it was shown that all three SNPs have a pronounced regulatory potential - they are located in DNA regions associated with histones marking enhancers and promoters in the binding sites of regulatory proteins - TBP, c-FOS, c-Jun and CTCF, in regions of regulatory DNA motifs, where binding to transcription factors occurs.

Keywords: matrix metalloproteinases, gene polymorphism, epigenetic factors, multifactorial pathology

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INTRODUCTION

In recent years, more scientific publications have been devoted to studies of genomic associations (GWAS), aimed at identifying the associations of single nucleotide polymorphisms (SNPs) with the development of various multifactorial diseases (Polonikov et al., 2017; Enkova et al., 2018; Ponomarenko et al., 2019 a,b). Therefore, the key task for the researcher is to identify and select for analysis polymorphic variants that can contribute to the estimated phenotype, that is, have significant regulatory potential (Ponomarenko et al., 2018). Polymorphic variants of matrix metalloproteinase (*MMP*) genes are promising candidates for the role of a molecular marker of sensitivity and the clinical course of a number of diseases. According to The Human Gene Database, *MMP* genes encode Zn-dependent proteases responsible for the degradation of extracellular matrix components, vasoactive mediators, and molecules involved in cell signaling (http://www.genecards.org/). It was previously shown that SNPs of metalloproteinases are associated with the development of ischemic stroke (Polonikov et al., 2019; Moskalenko et al., 2020) and arterial hypertension (AH) in residents of Central Russia (Moskalenko et al., 2019 a,b,c). However, the role of *MMP* in the formation of a predisposition to such multifactorial diseases as primary open-angle glaucoma (POAG) and peptic ulcer (PU) is not clear.

The objective of this study is to identify the associations of polymorphism of matrix metalloproteinase genes with multifactorial diseases and evaluate *in silico* their functional effects.

MATERIALS AND METHODS

The sample involved unconnected persons of Russian nationality, natives of the Central Black Earth Region of Russia: POAG - 932 people (patients n=536, control n=396), AH - 1405 people (patients n=939, control n=466), PU - 747 people (patients n=400, control n=347). The material for the study was genomic DNA isolated from venous blood samples by the standard

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Table 1. Distribution of polymorphic markers of matrix metalloproteinase genes associated with multifactorial diseases among patients and in control groups

SNP	Genotypes, alleles	POAG			AH			PU		
		Patients (n=536)	Control (n=396)	OR (95% CI) χ2, p	Patients (n=939)	Control (n=466)	OR (95% CI) χ2, p	Patients (n=400)	Control (n=347)	OR (95% CI) χ2, p
rs2250889 *MMP9*	Minor allele G	143 (13.52%)	75 (9.72%)	**1.45 (1.07-1.98) χ2 = 5.79; p=0.02**	271 (14.43%)	56 (16.29%)	0.74 (0.39-1.27) χ2=0.64; p=0.24	119 (14.87%)	95 (13. 69%)	1.32 (1.04-2.27) χ2 =1.67; p=0.54
	CC	401 (75.80%)	318 (82.38%)	**0.67 (0.48-0.94) χ2 = 5.36; p=0.02**	704 (74.97%)	335 (71.89%)	0.89 (0.67-1.19) χ2=0.58; p=0.45	301 (75.25%)	263 (75.79%)	1.01 (0.69-1.41) χ2 =0.001; p=1.00
	CG	113 (21.36%)	6 (15.80%)	**1.44 (1.01-2.07) χ2 = 4.12 p=0.04**	199 (21.19 %)	109 (23.39%)	0.76 (0.41-1.39) χ2=0.66; p=0.42	79 (19.75%)	73 (21.03%)	0.81 (0.39-1.46) χ2=0.39 p=0.51
	GG	15 (2.84%)	7 (1.81%)	1.58 (0.60-4.32) χ2 = 0.61; p=0.44	36 (3.84%)	22 (4.72%)	0.85 (0.68-1.07) χ2 =1.85; p=0.17	20 (5.00%)	11 (3.18%)	1.29 (0.61-3.28) χ2 = 0.52; p=0.39
	H_o/H_e (p)	0.21/0.23 (<0.05)	0.16/0.18 (>0.05)		0.31/0.32 (>0.05)	0.33/0.35 (>0.05)		0.48/0.49 (>0.05)	0.49/0.49 (>0.05)	
rs11568818 *MMP7*	Minor allele G	447 (41.70%)	336 (42.42%)	0.98 (0.80-1.20) χ2 =0.02; p=0.89	730 (39.29%)	403 (43.61%)	**0.84 (0.71-0.98) χ2 =4.61. p=0.03**	333 (41.42%)	296 (42.65%)	0.95 (0.72-1.25) χ2 = 1.10. p=0.75
	AA	187 (34.89%)	135 (34.09%)	1.03 (0.66-1.42) χ2 = 0.01; p=1.00	338 (36.38%)	147 (31.82%)	1.23 (0.96-1.56) χ2 =2.63. p=0.10	141 (35.25%)	116 (33.43%)	1.10 (0.77-1.56) χ2=0.20; p=0.65
	AG	251 (46.83%)	186 (46.97%)	1.00 (0.77-1.32) χ2 = 0.01; p=1.00	452 (48.65%)	227 (49.13%)	0.87 (0.67-1.12) χ2 = 1.13. p=0.29	189 (47.25%)	166 (47.84%)	0.97 (0.68-1.39) χ2 = 0.01; p=0.94
	GG	98 (18.28%)	75 (18.94%)	0.95 (0.63-1.44) χ2 = 0.02; p=0.90	139 (14.97%)	88 (19.05%)	**0.69 (0.49-0.97) χ2 = 4.63. p=0.03**	72 (17.50%)	65 (18.73%)	1.07 (0.58-1.99) χ2 = 0.01; p=0.93
	H_o/H_e (p)	0.47/0.48 (>0.05)	0.46/0.47 (>0.05)		0.49/0.48 (>0.05)	0.49/0.49 (>0.05)		0.37/0.38 (>0.05)	0.38/0.38 (>0.05)	
rs17576 *MMP9*	Minor allele C	441 (41.14%)	303 (38.26%)	1.13 (0.77-1.39) χ2 =0.18; p=0.84	715 (38.07%)	331 (35.51%)	1.14 (0.94-1.38) χ2 =1.84; p=0.18	328 (41.31%)	250 (36.13%)	**1.24 (1.00-1.54) χ2 =3.96; p=0.04**
	TT	198 (36.94%)	154 (38.39%)	0.78 (0.58-1.53) χ2 =1.88; p=0.13	375 (39.94%)	196 (42.06%)	0.88 (0.68-1.16) χ2 = 0.73. p=0.39	142 (35.80%)	142 (41.00%)	0.8 (0.59-1.09) χ2 =1.96; p=0.16
	TC	235 (43.84%)	181 (45.71%)	0.95 (0.79-1.15) χ2 =0.21; p=0.65	413 (43.98%)	209 (44.85%)	0.97 (0.74-1.27) χ2 = 0.03. p=0.86	182 (45.82%)	158 (45.73%)	1.01 (0.75-1.36) χ2 =0.001; p=1.00
	CC	103 (19.22%)	61 (15.40%)	1.17 (0.89-1.52) χ2=1.15; p=0.28	151 (16.08%)	61 (13.09%)	1.08 (0.81-1.45) χ2 =0.22; p=0.64	73 (18.44%)	46 (13.32%)	1.45 (0.97-2.24) χ2 =3.2; p=0.07
	H_o/H_e (p)	0.33/0.34 (>0.05)	0.34/0.34 (>0.05)		0.38/0.37 (>0.05)	0.39/0.38 (>0.05)		0.46/0.48 (>0.05)	0.46/0.46 (>0.05)	

phenol-chloroform extraction method. The rs2250889 MMP9, rs11568818 MMP7 and rs17576 MMP9 polymorphic loci were studied using the polymerase chain reaction on a CFX-96 Real-Time System thermal cycler (Bio-Rad Laboratories, Inc., US) using oligonucleotide primers and probes synthesized by Syntol LLC, Russia). The SNPs for the study were selected according to the criteria presented earlier (Ponomarenko et al., 2019 c,d).

To assess the compliance of the observed distribution of genotypes with the expected one, based on Hardy-Weinberg equilibrium, we used χ^2 test. The frequencies of alleles and genotypes in the studied patient and control samples were analyzed in 2x2 contingency tables using the χ2 test with Yates correction for continuity. The calculations were carried out in the STATISTICA for Windows 10.0. The orientation of associations of polymorphic loci with the development of AH was evaluated by the odds ratio (OR) and its 95% confidence interval (CI 95%).

The regulatory potential of single nucleotide polymorphisms was studied using HaploReg (v4.1) online program (http://archive.broadinstitute.org/mammals/haploreg/haploreg.php) (Ward et al., 2016).

RESULTS AND DISCUSSION

The analysis of the involvement of three single nucleotide polymorphisms of the matrix metalloproteinase genes - rs11568818 *MMP7*, rs2250889 *MMP9* and rs17576 *MMP9* in the formation of multifactorial diseases is carried out. Significant associations were established for the G allele and the CC and GC genotypes of rs2250889 *MMP9* – with primary open-angle glaucoma (p=0.02-0.04), for the G allele and the GG genotype of rs11568818 *MMP7* – with the development of AH (p=0.03), and for the C allele of rs17576 *MMP9* – with peptic ulcer (p=0.04) (**Table 1**).

Using HaploReg (v4.1), it was established that all the SNPs studied have significant regulatory potential. The rs2250889 *MMP9* polymorphic locus is located in the DNA region associated with histones marking enhancers and promoters in 18 and 11 different organs and tissues, respectively, including in brain structures, neuronal precursor cells, mesenchymal and hematopoietic stem cells. The polymorphic marker rs2250889 is located in the binding site of the regulatory CTCF protein and in regions of regulatory DNA motifs in which binding to 2 transcription factors occurs. At the same time, the G allele, which is a risk factor in the development of POAG, reduces the affinity for the NRSF_disc3 transcription factors (the difference

between ΔLOD scores of the alternative and reference alleles is 4.6) and NRSF_known2 (ΔLOD scores = 11.4). We have found associations of the rs2250889 polymorphic variant with the development of primary open-angle glaucoma, which is consistent with published data. Thus, the study of the Chinese population found G allele to be significantly more common in the group of patients with POAG compared with the control group (OR=1.11, p<0.001) (Zhao et al., 2019). The rs11568818 *MMP7* polymorphic variant is located in the region of modified histones (H3K4me1 and H3K4me3), which mark enhancers and promoters in 11 different organs and tissues, including peripheral blood cells, endotheliocytes, mesenchymal stem cells, etc. It was revealed that rs11568818 is located in the binding sites of regulatory proteins - TBP (TATA-binding protein), c-FOS, c-Jun and in regions of regulatory DNA motifs in which binding with 4 transcription factors occurs. Moreover, the G allele increases the affinity for the transcription factors - Foxa known1 (ΔLOD scores = -3.1), PLZF (ΔLOD scores = -1.5), Pou5f1 known2 (ΔLOD scores = -3.2) and decreases the affinity for the transcription factor GR known4 (LOD scores = 0.8) It was previously shown that SNP s11568818 is associated with the development of hypertension in women (OR=1.49, p=0.002), which is consistent with the results (Moskalenko et al., 2018). Th rs17576 MMP9e polymorphic variant is located in the DNA region associated with histones marking promoters and enhancers in the duodenum, esophagus, stomach, pancreas, peripheral blood, etc. (17 different tissues and organs). This SNP is located in regions of DNA where binding to the Pax-4_1 transcription factor occurs, while the G allele increases affinity for this factor (ΔLOD scores = -2.1). Our data are consistent with the results obtained by E. Shaymardanova et al. (2016) when studying the population of the Bashkir Tatars, where rs17576 associations with the development of ulcer were also established (p<0.05). The associations we established with SNPs with the development of multifactorial diseases can be associated both with the regulatory effects of the SNPs described above and with the biological functions of the corresponding metalloproteinases. Matrix metalloproteinases are capable of cleaving almost all substrates in the extracellular matrix, which determines their influence on proliferation, remodeling, repair, apoptosis, and determines their participation in biological processes both in normal and pathological conditions (Mocchegiani et al., 2011; Adda et al, 2016).

CONCLUSION

Thus, this study revealed the involvement of polymorphic loci of *MMP* genes in the development of primary open-angle glaucoma (rs2250889 *MMP9*, p=0.02-0.04), arterial hypertension (rs11568818 *MMP7*, p=0.03) and peptic ulcer (rs17576 *MMP9*, p=0.04). *In silico* tests established the functional effects of these SNPs - all three variants are located in DNA regions associated with histone proteins that mark promoters and enhancers in various tissues, and are binding sites to transcription factors and regulatory proteins. Our data are consistent with the biological effects of metalloproteinases and previous studies, and indicate the association of *MMP* genes with the development of multifactorial diseases.

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