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HUMAN GENETICS

Gender-Specific Features of Associations of Polymorphism of Matrix Metalloproteinase Genes with the Development of Peptic Ulcer Disease in the Population of the Central Chernozem Region of Russia

O. N. Minyaylo[a], I. V. Ponomarenko[a], and M. I. Churnosov[a, *]

[a] *Belgorod State University, Belgorod, 308015 Russia*

**e-mail: churnosov@bsu.edu.ru*

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Abstract—Gender-specific features of associations of functionally significant polymorphic loci of matrix metalloproteinase genes with the development of peptic ulcer disease (PUD) in the population of the European part of Russia were studied. The study included 305 men (patients with PUD—188, control group—117) and 441 women (patients with PUD—211, control group—230). Ten functionally significant polymorphic loci of matrix metalloproteinase genes were genotyped: rs1799750 *MMP1*, rs243865 *MMP2*, rs679620 *MMP3*, rs1940475 *MMP8*, rs3918242, rs3918249, rs17576, rs3787268, rs2250889, rs17577 of the *MMP9* gene. Associations of polymorphic loci of *MMP* genes with PUD were studied by logistic regression (dominant, recessive, and additive genetic models of allele interaction were evaluated). It was found that alleles *C* rs3918249 (OR = 1.61, $p_{perm} = 0.048$) and *G* rs17576 (OR = 1.48–2.08, $p_{perm} \leq 0.042$) of the *MMP9* gene were risk factors for the occurrence of PUD in men. In women, the *2G* rs1799750 allele of the *MMP1* gene had a protective value for the development of the disease (OR = 0.74, $p_{perm} = 0.047$). Polymorphic loci associated with PUD in men and women exhibit pronounced epigenetic effects (they affect the affinity of DNA motifs for various transcription factors, are located in the region of promoters and enhancers in the stomach and duodenum, and are associated with gene expression in various parts of the digestive system). Gender-specific features of associations of polymorphism of *MMP* genes with the development of PUD were revealed: rs3918249 and rs17576 of the *MMP9* gene determine the susceptibility to the development of PUD in men, and rs1799750 of the *MMP1* gene determines the susceptibility in women.

Keywords: peptic ulcer disease, matrix metalloproteinases, polymorphism, associations, gender

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INTRODUCTION

Peptic ulcer disease (PUD) of the stomach and duodenum still remain one of the most frequent diseases of the digestive system throughout the world [1]. In the United States, up to 500000 new cases of PUD are registered annually [2]. According to the official statistics provided by Federal State Statistics Service of Russia for 2018, the diagnosis of stomach and duodenum PUD was registered in 834.8 out of 100000 Russian citizens. However, in the last years, a certain downward trend in the frequency of stomach and duodenum PUD have been observed from 95.6 cases of first established diagnosis per 100000 patients in 2010 to 71.9 cases per 100000 patients in 2018 [3]. It was shown that the frequency of PUD is characterized by gender-associated features. It is believed that men suffer the duodenum PUD more often, while among the patients with stomach PUD the ratio of women and men is almost equal [4].

According to the modern conceptions of etiology and pathogenesis of PUD, the development of the disease is associated with imbalance between the aggressive factors (acidic-peptic content of stomach) and protective mechanisms of mucous tunic of the stomach and duodenum [4]. Presently, it is believed that the central role in the development of PUD belongs to the bacteria *H. pylori* [5]. These bacteria produce a variety of enzymes, such as ureases, proteases and phospholipases, which damage the protective coat of the mucous tunic. *H. pylori* also produces cytotoxins, which induce the release of various cytokines (tumor necrosis factors, interleukins, etc.) from the mucous tunic of stomach that initiate inflammation [4, 5]. It has been reported that about 80% of duodenum ulcers and 60% of stomach ulcers are associated with *H. pylori* [6].

It is noteworthy that inflammation in the mucous tunic of the stomach and duodenum causes the involvement of matrix metalloproteinases (MMPs) in the pathogenesis of PUD, which play the key role in the intercellular matrix degradation and remodeling during inflammation and recovery of injuries and also

during the infection with *H. pylori* [7, 8]. Several studies demonstrated increased levels of matrix metalloproteinases (MMP3, MMP9, etc.) in the ulcer area. It was also shown that the level of MMPs correlated with the levels of IL1β, IL6, and IL8, and the effective treatment of PUD was associated with a decrease in the level of MMPs [9–13]. It is important to emphasize that one of the key roles in the induction of the MMP synthesis belongs to *H. pylori* [11, 12]. A significant association between the *MMP* gene polymorphism and susceptibility to the PUD has been demonstrated [13, 14]. However, it should be noted that only one study of the association of the polymorphism of the *MMP* genes with the development of PUD has been carried out in Russia, in the population of Bashkortostan [14]. Such a small level of studies shows the necessity of further molecular-genetic investigations of PUD in different ethnic and geographical populations of Russia, which would be aimed also at the revelation of gender-associated features of the involvement of the polymorphism of *MMP* genes in the development of PUD.

The goal of the present study was to analyze the gender-specific associations between the polymorphic loci of matrix metalloproteinase genes with the formation of stomach and duodenum PUD in the population of the Central Chernozem region of Russia.

MATERIALS AND METHODS

The study included 746 individuals: 305 men (PUD patients—188, control group—117) and 441 women (PUD patients—211, control group—230). Among women, 149 suffered stomach PUD (70.61%) and 62 suffered duodenum PUD (29.39%). Among men, suffering PUD, these numbers were 68 (36.17%) and 120 (63.83%), respectively. All individuals chosen for the study were ethnically Russians, lived in the Central Chernozem region of Russia [15, 16], and were not relatives of one another. The study included only the patients whose diagnosis was confirmed clinically, as well as by the methods of laboratory diagnostics [1]. The diagnostics of PUD was performed in the Department of Gastroenterology of the St. Joseph Regional Clinical Hospital in Belgorod. All patients underwent endoscopic study of the stomach and duodenum (gastroduodenoscopy) followed by biopsy. Morphological study of the biopsy samples was carried out in order to reveal *H. pylori*. *H. pylori* was identified in 202 PUD patients (50.62% of patients), including 100 men (53.19% of all male PUD patients) and 102 women (48.34% of all female PUD patients). The control group included healthy individuals of the same gender and age as the patients without clinical and endoscopic traits of PUD. The average age of male PUD patients and the male control group was 48.24 ± 13.63 and 48.64 ± 13.52 years, respectively ($p > 0.05$). The average age of female PUD patients and the female control group was 49.04 ± 12.53 and 49.23 ± 12.75 years, respectively. The study was carried out under the control of Ethics Committee of the Medical Institute of Belgorod State National Research University. All individuals provided a signed informed consent to participate in the study.

The material for the molecular-genetic study was composed of DNA samples, which were isolated from peripheral blood by phenol-chloroform extraction. Ten polymorphic loci of matrix metalloproteinase genes were genotyped: rs1799750 *MMP1*, rs243865 *MMP2*, rs679620 *MMP3*, rs1940475 *MMP8*, rs3918242, rs3918249, rs17576, rs3787268, rs2250889, and rs17577 of the *MMP9* gene. The loci included in the study are regulatory single nucleotide polymorphisms (rSNP) [17, 18]. According to the HaploReg (v4.1.) database (http://archive.broadinstitute.org/mammals/haploreg/haploreg.php), they possess a significant regulatory potential. The amplification of polymorphic loci was performed by polymerase chain reaction in the CFX96 amplifier by the method of TaqMan probes, using specially designed kits (Test-Gene, Ltd., Russia).

The association of the loci of *MMP* genes with PUD was assessed by the method of logistic regression (dominant, recessive, and additive genetic models of allele interaction were evaluated), using the PLINK v. 2.050 program (http://pngu.mgh.harvard.edu/Èpurcell/plink). The association features were assessed by the parameter of odds ratio (OR) and its 95% confidence interval (95% OR). The correction for multiple comparisons was performed with adaptive permutation test (p_{perm}). The test was considered to be statistically significant at $p_{perm} < 0.05$.

Epigenetic effects were studied for the polymorphic *MMP* loci, which demonstrated significant association with PUD. This included use of the HaploReg (http://archive.broadinstitute.org/mammals/haploreg/haploreg.php) and the GTExportal (http://www.gtexportal.org/) databases as described in [19–22].

RESULTS AND DISCUSSION

The distributions of genotype and allele frequencies in the studied groups of men and women suffering PUD, as well as the control group and in the whole sample, are shown in the Table 1. The observed distribution of genotypes corresponded to the expected one in accordance with the Hardy–Weinberg principle for all ten loci of *MMP* genes studied ($p_{bonf} > 0.05$).

Features of association of the polymorphic loci of *MMP* genes with development of PUD in men and women have been revealed (Table 2). It was shown that two polymorphic loci of the *MMP9* gene (rs3918249 and rs17576) were associated with the disease in men, whereas in women the rs1799750 polymorphic locus of the *MMP1* gene was shown to be associated with PUD. In men, the risk factors for the development of PUD were the alleles *C* rs3918249 (according to the

Table 1. Frequencies of the polymorphic *MMP* alleles and genotypes in the studied groups of men and women suffering PUD and in the control group

Locus	Genotype, minor allele, correspondence to the HWE	Women ($n = 441$)		Men ($n = 305$)		Total ($n = 746$) % (n)
		control ($n = 230$) % (n)	sick ($n = 211$) % (n)	control ($n = 117$) % (n)	sick ($n = 188$) % (n)	
rs1940475 *MMP8*	*CC*	26.52 (61)	32.23 (68)	30.17 (35)	25.00 (46)	28.34 (210)
	CT	45.65 (105)	41.71 (88)	45.69 (53)	49.46 (91)	45.48 (337)
	TT	27.83 (64)	26.06 (55)	24.14 (28)	25.54 (47)	26.18 (194)
	T	50.65	46.92	46.98	50.27	48.92
	p_{HWE}	0.19	0.02	0.36	0.88	0.02
rs1799750 *MMP1*	*1G1G*	28.89 (65)	37.62 (76)	29.82 (34)	24.72 (45)	30.43 (220)
	1G2G	44.44 (100)	43.56 (88)	48.25 (55)	50.56 (92)	46.33 (335)
	2G2G	26.67 (60)	18.81 (38)	21.93 (25)	24.72 (45)	23.24 (168)
	2G	48.89	40.59	46.05	50.00	46.40
	p_{HWE}	0.11	0.19	0.85	1.00	0.07
rs679620 *MMP3*	*TT*	26.52 (61)	29.81 (62)	24.35 (28)	19.89 (37)	25.71 (190)
	TC	48.69 (112)	47.11 (98)	50.43 (58)	50.00 (93)	48.85 (361)
	CC	24.78 (57)	23.08 (48)	25.22 (29)	30.11 (56)	25.44 (188)
	C	49.13	46.63	50.43	55.11	49.86
	p_{HWE}	0.69	0.46	1.00	1.00	0.55
rs243865 *MMP2*	*CC*	61.40 (140)	57.77 (119)	48.69 (56)	55.92 (104)	57.24 (419)
	CT	31.58 (72)	35.44 (73)	44.35 (51)	34.95 (65)	35.66 (261)
	TT	7.02 (16)	6.79 (14)	6.96 (8)	7.53 (14)	7.10 (52)
	T	22.81	24.51	29.13	25.41	24.93
	p_{HWE}	0.13	0.57	0.50	0.43	0.20
rs3918242 *MMP9*	*CC*	68.72 (156)	69.71 (145)	70.69 (82)	67.94 (125)	69.12 (508)
	CT	28.19 (64)	27.40 (57)	25.86 (30)	31.52 (58)	28.43 (209)
	TT	3.08 (7)	2.89 (6)	3.45 (4)	0.54 (1)	2.45 (18)
	T	17.18	16.59	16.38	16.30	16.67
	p_{HWE}	0.82	0.81	0.50	0.06	0.59
rs3918249 *MMP9*	*TT*	37.83 (87)	32.68 (67)	47.83 (55)	36.22 (67)	37.55 (276)
	TC	43.48 (100)	53.66 (110)	40.00 (46)	48.65 (90)	47.07 (346)
	CC	18.69 (43)	13.66 (28)	12.17 (14)	15.13 (28)	15.38 (113)
	C	40.43	40.49	32.17	39.46	38.91
	p_{HWE}	0.13	0.15	0.39	0.88	0.82
rs17576 *MMP9*	*AA*	37.83 (87)	34.60 (73)	47.41 (55)	37.10 (69)	38.22 (284)
	AG	47.39 (109)	47.87 (101)	42.24 (49)	43.55 (81)	45.76 (340)
	GG	14.78 (34)	17.53 (37)	10.35 (12)	19.35 (36)	16.02 (119)
	G	38.48	41.47	31.47	41.13	38.90
	p_{HWE}	1.00	0.89	0.83	0.17	0.32

Table 1. (Contd.)

Locus	Genotype, minor allele, correspondence to the HWE	Women ($n = 441$)		Men ($n = 305$)		Total ($n = 746$) % (n)
		control ($n = 230$) % (n)	sick ($n = 211$) % (n)	control ($n = 117$) % (n)	sick ($n = 188$) % (n)	
rs3787268 *MMP9*	*GG*	59.65 (136)	58.57 (123)	68.38 (80)	60.87 (112)	61.03 (451)
	GA	35.96 (82)	37.62 (79)	28.20 (33)	34.78 (64)	34.91 (258)
	AA	4.39 (10)	3.81 (8)	3.42 (4)	4.35 (8)	4.06 (30)
	A	22.37	22.62	17.52	21.74	21.52
	p_{HWE}	0.70	0.33	0.75	1.00	0.39
rs2250889 *MMP9*	*CC*	76.55 (173)	80.86 (169)	81.03 (94)	83.16 (153)	80.14 (589)
	CG	20.80 (47)	16.75 (35)	16.38 (19)	16.30 (30)	17.82 (131)
	GG	2.65 (6)	2.39 (5)	2.59 (3)	0.54 (1)	2.04 (15)
	G	13.05	10.77	10.78	8.70	10.95
	p_{HWE}	0.23	0.07	0.12	1.00	0.02
rs17577 *MMP9*	*GG*	68.75 (154)	69.42 (143)	70.69 (82)	66.67 (120)	68.73 (499)
	AG	27.23 (61)	28.15 (58)	25.86 (30)	32.22 (58)	28.51 (207)
	AA	4.02 (9)	2.43 (5)	3.45 (4)	1.11 (2)	2.76 (20)
	A	17.63	16.50	16.38	17.22	17.01
	p_{HWE}	0.36	1.00	0.50	0.11	0.89

dominant model, OR = 1.61, 95% OR 1.01–2.59, $p = 0.047$, $p_{perm} = 0.048$) and *G* rs17576 (according to the additive model, OR = 1.48, 95% OR 1.06–2.07, $p = 0.023$, $p_{perm} = 0.024$; according to the recessive model, OR = 2.08, 95% OR 1.03–4.19, $p = 0.040$, $p_{perm} = 0.042$). In women, the *2G* rs1799750 allele of the *MMP1* gene demonstrated a protective effect during the development of the disease in accordance with the additive model (OR = 0.74, 95% OR 0.57–0.96, $p = 0.021$, $p_{perm} = 0.047$). Hence, according to the data obtained, the rs3918249 and rs17576 polymorphisms of the *MMP9* gene determine the susceptibility to PUD in men, and the rs1799750 polymorphism of the *MMP1* gene determines the susceptibility to PUD in women.

The epigenetic analysis of polymorphic loci involved in the formation of PUD in men and women showed the following results. First, the rs3918249 and rs17576 loci of the *MMP9* gene were shown to affect the affinity of DNA motifs for four (Hmx, Hoxb8, Arid3a, Pax-5) and one (Pax-4) of the transcription factors, respectively. Moreover, the allelic variations of these loci associated with increased risk of PUD in men (the *C* rs3918249 and *G* rs17576 alleles) determine the increased affinity for the Hmx (the ΔLOD score for the *C* and *T* rs3918249 alleles is 1.9) and Hoxb8 (ΔLOD = 2.9) transcription factors and low affinity of DNA motives for the Arid3a (ΔLOD = –0.7), Pax-5 (ΔLOD = –3.9), and Pax-4 (the ΔLOD score for the alleles *G* and *A* rs3918249 is –2.1).

Second, the polymorphic locus rs3918249 is located in the region of histone proteins, which mark regulatory regions (promoters and enhancers) of the *MMP9* gene in the mucous tunic of the stomach (H3K4me1_Enh) and duodenum (H3K4me3_Pro), and in the smooth muscle of the stomach (H3K4me3_Pro) and duodenum (H3K4me1_Enh and H3K4me3_Pro) of an adult person. The expressive regulatory potential was shown for the rs17576, which is located near the promoter and enhancer regions of the *MMP9* gene in different parts of the digestive system of both fetus (stomach—H3K4me1_Enh) and adult: stomach (H3K4me1_Enh) and duodenum (H3K4me1_Enh and H3K4me3_Pro) mucous tunic, and stomach (H3K4me1_Enh and H3K4me3_Pro) and duodenum (H3K4me1_Enh and H3K4me3_Pro) smooth muscle.

Third, the rs3918249 single nucleotide polymorphism of the *MMP9* gene was associated with expression of several genes (*PLTP*, *NEURL2*, and *RP3-337O18.9*) in different parts of the digestive system: stomach (*PLTP*, ($\beta = -0.25$, $p = 1.7 \times 10^{-5}$, $p_{FDR} \leq 0.05$), esophagus (*PLTP*, $\beta = -0.13$, $p = 6.2 \times 10^{-5}$, $p_{FDR} \leq 0.05$), and large intestine (*PLTP*, $\beta = -0.25$, $p = 1.7 \times 10^{-5}$, $p_{FDR} \leq 0.05$; *NEURL2*, $\beta = -0.22$, $p =$

Table 2. Comparative analysis of associations between the polymorphism of *MMP* genes and development of PUD in men and women

Polymorphism	Gene	Rare allele	*n*	Additive model				Dominant model				Recessive model			
				OR	95% OR		*p*	OR	95% OR		*p*	OR	95% OR		*p*
					L95	U95			L95	U95			L95	U95	
Men															
rs1940475	*MMP-8*	*T*	300	1.14	0.82	1.57	0.442	1.30	0.77	2.18	0.326	1.08	0.63	1.85	0.784
rs1799750	*MMP-1*	*2G*	296	1.17	0.84	1.63	0.352	1.29	0.77	2.19	0.335	1.17	0.67	2.04	0.582
rs679620	*MMP-3*	*T*	301	0.83	0.59	1.15	0.263	0.78	0.46	1.32	0.360	0.77	0.44	1.35	0.362
rs243865	*MMP-2*	*T*	298	0.83	0.57	1.02	0.321	0.72	0.45	1.15	0.171	1.11	0.45	2.73	0.824
rs3918242	*MMP-9*	*T*	300	0.99	0.63	1.58	0.980	1.14	0.69	1.89	0.616	0.15	0.02	1.39	0.095
rs3918249	*MMP-9*	*C*	300	1.37	0.97	1.93	0.076	**1.61**	**1.01**	**2.59**	**0.047**	1.29	0.65	2.56	0.473
rs17576	*MMP-9*	*G*	302	**1.48**	**1.06**	**2.07**	**0.023**	1.53	0.96	2.45	0.077	**2.08**	**1.03**	**4.19**	**0.040**
rs3787268	*MMP-9*	*A*	301	1.31	0.86	1.99	0.209	1.39	0.85	2.27	0.187	1.28	0.38	4.36	0.689
rs2250889	*MMP-9*	*G*	300	0.80	0.47	1.37	0.410	0.87	0.47	1.58	0.640	0.21	0.02	2.00	0.173
rs17577	*MMP-9*	*A*	296	1.07	0.68	1.68	0.783	1.21	0.73	2.00	0.468	0.31	0.06	1.75	0.186
Women															
rs1940475	*MMP-8*	*T*	441	0.88	0.68	1.12	0.297	0.76	0.50	1.15	0.189	0.91	0.60	1.39	0.678
rs1799750	*MMP-1*	*2G*	427	**0.74**	**0.57**	**0.96**	**0.021**	0.67	0.45	1.01	0.056	0.64	0.40	1.01	0.055
rs679620	*MMP-3*	*T*	438	0.91	0.70	1.18	0.469	0.85	0.56	1.29	0.445	0.91	0.59	1.41	0.676
rs243865	*MMP-2*	*T*	434	1.09	0.81	1.48	0.568	1.16	0.79	1.71	0.441	0.97	0.46	2.03	0.928
rs3918242	*MMP-9*	*T*	435	0.96	0.67	1.37	0.816	0.95	0.64	1.44	0.823	0.93	0.31	2.82	0.903
rs3918249	*MMP-9*	*C*	435	1.00	0.76	1.32	0.987	1.25	0.84	1.86	0.263	0.69	0.41	1.16	0.157
rs17576	*MMP-9*	*G*	441	1.13	0.86	1.48	0.367	1.15	0.78	1.70	0.481	1.23	0.74	2.04	0.433
rs3787268	*MMP-9*	*A*	438	1.02	0.73	1.41	0.927	1.05	0.71	1.53	0.819	0.86	0.33	2.23	0.762
rs2250889	*MMP-9*	*G*	435	0.82	0.55	1.22	0.324	0.77	0.49	1.23	0.274	0.90	0.27	2.99	0.862
rs17577	*MMP-9*	*A*	430	0.93	0.65	1.32	0.664	0.97	0.64	1.46	0.881	0.59	0.20	1.80	0.358

OR—odds ratio, 95% OR—confidence interval of the OR (L95—lower limit, U95—upper limit), *p*—significance level; bold font indicates statistically significant values, taking into account the premutational test results (1000 permutations were carried out).

4.9×10^{-5}, $p_{FDR} \le 0.05$; *RP3-337O18.9*, $\beta = -0.28$, $p = 3.5 \times 10^{-6}$, $p_{FDR} \le 0.05$).

The rs17576 polymorphism of the *MMP9* gene was also characterized by important eQTL value in different parts of the digestive system. It was shown to be associated with expression of three genes (*PLTP*, *NEURL2*, *RP3-337O18.9*) in the esophagus (*PLTP*, $\beta = -0.15$, $p = 2.7 \times 10^{-6}$, $p_{FDR} \le 0.05$; *NEURL2*, $\beta = -0.25$, $p = 1.8 \times 10^{-5}$, $p_{FDR} \le 0.05$; *RP3-337O18.9*, $\beta = -0.23$, $p = 7.4 \times 10^{-5}$, $p_{FDR} \le 0.05$) and large intestine (*PLTP*, $\beta = -0.18$, $p = 5.6 \times 10^{-7}$, $p_{FDR} \le 0.05$; *NEURL2*, $\beta = -0.23$, $p = 1.3 \times 10^{-5}$, $p_{FDR} \le 0.05$; *RP3-337O18.9*, $\beta = -0.28$, $p = 1.9 \times 10^{-10}$, $p_{FDR} \le 0.05$). It is noteworthy that allelic variations of these loci associated with increased risk of PUD in men (the *C* rs3918249 and *G* rs17576 alleles) are associated with decreased expression ($\beta < 0$) of all three genes examined above (*PLTP*, *NEURL2*, *RP3-337O18.9*) in different parts of the digestive tract.

Fourth, the expressed epigenetic effects were also typical of the polymorphic rs1799750 locus of the *MMP1* gene, which determines the susceptibility to PUD in women. It was shown to affect the affinity of DNA motifs for 21 transcription factors (AP-1, CHX10, DMRT2, Hoxb4, PLZF, etc.) and is located near the modified histone proteins, which mark the enhancers (H3K4me1_Enh) and "active" enhancers (H3K27ac_Enh) in the stomach and small intestine of fetus and in the mucous tunic of the stomach (H3K4me1_Enh) and duodenum (H3K4me1_Enh) and smooth muscle of the stomach (H3K27ac_Enh) and duodenum (H3K27ac_Enh) of adult. It is also located in the region of interaction with two regulatory proteins: CFOS and GATA2. It should be noted that the *2G* rs1799750 allele of the *MMP1* gene, which is associated with low risk of PUD in women, increases the affinity of DNA ($\Delta LOD > 0$) only for six transcription factors (AP-1, Dbx1, En-1, Evi-1, HMG-IY, Pou3f2), though it decreases it for the majority (16) of

transcription factors (CHX10, DMRT2, Hoxb4, PLZF, Pax-4, Pax-6, etc.) ($\Delta LOD < 0$).

Fifth, the rs1799750 polymorphism of the *MMP1* gene is associated with transcriptional activity of this gene in the digestive system (esophagus) ($\beta = -0.30$, $p = 2.8 \times 10^{-7}$, $p_{FDR} \le 0.05$). At the same time, the *2G* rs1799750 allele, which provides a protective effect in women, is associated with increased expression of the *MMP1* gene (*1G* $\beta < 0$ for the reference allele).

Hence, the data obtained demonstrated intense epigenetic effects of polymorphic rs3918249 and rs17576 loci of the *MMP9* gene and the rs1799750 locus of the *MMP1* gene. These effects were manifested in different parts of the digestive system, including the target organs, which undergo injury at PUD in the stomach and duodenum. It is noteworthy that polymorphic rs3918249 and rs17576 loci of the *MMP9* gene are located at a distance of 2.1 kb from each other and are linked tightly ($r^2 = 0.99$, $D' = 0.99$). Therefore, their functional effects may overlap.

It should be noted that only single publications are presently available. These studies deal with the role of the *MMP* gene polymorphisms in the pathogenesis of PUD [13, 14, 23, 24], and only one of these studies was carried out in Russia. For example, Shaymardanova et al. [14] studied the associations of the three polymorphic *MMP* loci studied in our work (rs1799750 of *MMP1*, rs3918242 and rs17576 of *MMP9*) with PUD in the population of Bashkortostan. For two of three polymorphic loci, the authors revealed the associations with the disease: the *1G/2G* rs1799750 genotype of the *MMP1* gene was the risk factor for the development of PUD in the population of Tatars (OR = 1.94, $p = 0.02$), while the rs17576 locus of the *MMP9* gene was associated with the development of PUD in the population of Tatars (OR = 0.49, $p = 0.007$ for the *A/A* genotype and OR = 2.19, $p = 0.003$ for the *A/G* genotype), the formation of PUD in the duodenum (OR = 1.57, $p = 0.009$ for the *A/G* genotype), and the development of PUD in Tatar population infected with *H. pylori* (OR = 0.54, $p = 0.03$ for the *A/A* genotype and OR = 2.23, $p = 0.009$ for the *A/G* genotype).

The data on the risk of PUD development in men carrying the *G* rs17576 allele and subsequently the protective role of the reference A allele are fully consistent with the data of Shaymardanova et al. [14], though they contradictory with the data of Yeh et al. [13] and Hellmig et al. [23]. Yeh et al. [13] did not reveal any associations between rs17576 *MMP9* and development of *H. pylori*-positive PUD of the stomach and duodenum in the population of Taiwan, whereas the study of Hellmig et al. [23], which was carried out in Germany, conversely, revealed the association of the *A* rs17576 allele with the risk of *H. pylori*-positive PUD of the stomach. As in our study, Shan et al. [24] did not find statistically significant associations between the rs3918242 allele of the *MMP9* gene and formation of PUD in the duodenum of children in the Chinese population.

Numerous studies showed the connection between the *MMP1* and *MMP9* polymorphic loci and the development of oncological diseases of the digestive system [25–33]. Most data confirming the involvement of these polymorphisms in the formation of malignant tumors were obtained for the rs1799750 allele of the *MMP1* gene. This polymorphism was shown to be associated with cancer of the esophagus (esophageal adenocarcinoma) [25–28] and stomach cancer [29–32]. The study of Okada et al. [33] showed the association between rs17576 *MMP9* and stomach cancer both independently and within the CAA rs3918242–rs17576–rs17577 *MMP9* haplotype. Therefore, it may be concluded that there are common genetic mechanisms (in the form of functionally significant polymorphic loci of the *MMP1* and *MMP9* genes), which determine susceptibility to both ulcer disease and carcinogenesis in the digestive system.

Gender-related associations between the *MMP* polymorphisms and the development of PUD have been found. Indeed, rs3918249 and rs17576 of the *MMP9* gene determine the susceptibility of men to the development of PUD, and rs1799750 of the *MMP1* gene determines the same in women. The *C* rs3918249 (OR = 1.61, $p_{perm} = 0.048$) and *G* rs17576 (OR = 1.48–2.08, $p_{perm} \le 0.042$) alleles of the *MMP9* genes are considered to be the risk factors for PUD in men. In women, the *2G* rs1799750 allele of the *MMP1* gene (OR = 0.74, $p_{perm} = 0.047$) demonstrated a protective effect. The polymorphic loci associated with PUD in men and women demonstrated obvious epigenetic effects (they affect the affinity of DNA motifs for a variety of transcription factors, are located near the promoters and enhancers in the stomach and duodenum, and are connected with the expression of genes in different parts of the digestive system).

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare no conflict of interest.

Statement of compliance with standards of research involving humans as subjects. All experimental procedures involving the participation of people were carried out in accordance with ethical standards of the Institutional and/or National Committee for Scientific Ethics, as well as with the Declaration of Helsinki (1964) and its following changes or comparable ethical standards.

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