RESEARCH ARTICLE

Association of the functionally significant polymorphisms of the *MMP9* gene with *H. pylori*-positive gastric ulcer in the Caucasian population of Central Russia

Volodymyr Dvornyk[1], **Irina Ponomarenko**[2], **Oksana Minyaylo**[2], **Evgeny Reshetnikov**[2]*, **Mikhail Churnosov**[2]

1 Department of Life Sciences, College of Science and General Studies, Alfaisal University, Riyadh, Saudi Arabia, 2 Department of Medical Biological Disciplines, Belgorod State University, Belgorod, Russia

* reshetnikov@bsu.edu.ru

Abstract

Background and purpose

The study analyzed the association of functionally significant polymorphisms of matrix metalloproteinases (*MMPs*) genes with the development of gastric ulcer (GU) in Caucasians from Central Russia.

Methods

The 781 participants, including 434 patients with GU (196 *Helicobacter pylori* (*H. pylori*)-positive and 238 *H. pylori*-negative) and 347 controls (all *H. pylori*-negative) were recruited for the study. Ten SNPs of the *MMP1* (rs1799750), *MMP2* (rs243865), *MMP3* (rs679620), *MMP8* (rs1940475), and *MMP9* (rs3918242, rs3918249, rs3787268, rs17576, rs17577, and rs2250889) genes were considered for association with GU using multiple logistic regression. The SNPs associated with GU and loci linked ($r^2 \geq 0.8$) to them were analyzed *in silico* for their functional assignments.

Results

The SNPs of the *MMP9* gene were associated with *H. pylori*-positive GU: alleles C of rs3918249 (OR = 2.02, p_{perm} = 0.008) and A of rs3787268 (OR = 1.60–1.82, $p_{perm} \leq$ 0.016), and eight haplotypes of all studied *MMP9* gene SNPs (OR = 1.85–2.04, $p_{perm} \leq$ 0.016) increased risk for *H. pylori*-positive GU. None of the analyzed SNPs was independently associated with GU and *H. pylori*-negative GU. Two haplotypes of the *MMP9* gene (contributed by rs3918242, rs3918249, rs17576, and rs3787268) increased risk for GU (OR = 1.62–1.65, $p_{perm} \leq$ 0.006). Six loci of the *MMP9* gene, which are associated with *H. pylori*-positive GU, and 65 SNPs linked to them manifest significant epigenetic effects, have pronounced eQTL (17 genes) and sQTL (6 genes) values.

Conclusion

SNPs of the *MMP9* were associated with *H. pylori*-positive GU but not with *H. pylori*-negative GU in Caucasians of Central Russia.

Introduction

Gastric ulcer (GU), a disease occurring in the stomach mucosa, is the mucosal inflammation and necrotic lesion that extend to the underlying smooth muscles and are caused by multiple pathogenic factors [1]. GU is a common disease affecting about 10% of population [2]. Common causes of GU include *Helicobacter pylori* (*H. pylori*) infection (70–80% of GU patients), intake of non-steroidal anti-inflammatory drugs (NSAIDs), and the digestion of the gastric mucous by gastric acid/pepsin [3, 4].

The development of GU is a complex process that involves secretion of acids, generation of the reactive oxygen species, inhibition of prostaglandins, and degradation of the extracellular matrix (ECM) [5]. Damage of gastric mucosa is associated with ECM degradation in which matrix metalloproteinases (MMPs) play a key role [6]. MMPs are calcium-dependent endopeptidases involved in various processes, including ECM remodeling, cell proliferation, and inflammation. MMPs are synthesized and secreted by gastric epithelial cells, neutrophils, and macrophages [7]. Remodeling of the ECM by MMPs is thought to be one of the important factors contributing to gastric ulceration [8, 9]. Several animal studies were focused on the role of MMPs in GU [9–11]. There is evidence that MMP9 is important in the early stage of chronic GU [12].

Genetic variation in the *MMP* genes may be an important element of a complex genetic risk profile that determines the development of GU in chronic *H. pylori* infection [13]. *H. pylori* infection can increase the MMP3, MMP7, and MMP9 levels in the gastric mucosa and sera [14–16]. The significantly higher levels of the MMP9 protein were observed in *H. pylori*-positive GU than in the *H. pylori*-negative one [17].

Despite the solid evidence for the important role of MMP in GU, an association of MMP polymorphisms with GU has been studied poorly: there are only very few studies on this problem [13, 18]. The lack of experimental evidence about the possible association of the *MMPs* with GU prompts for filling this gap.

This study analyzed polymorphisms of the *MMP1*, *MMP2*, *MMP3*, *MMP8*, and *MMP9* genes for the association and possible role in the development of GU in a Caucasian sample from Central Russia.

Materials and methods

Study subjects

Given the available data about the allele frequencies of the *MMP* gene polymorphisms in patients with GU and controls [13], we calculated that sample size of 700 should be sufficient to ensure the statistical power of 0.80 at α = 0.05 significance level. In total, 781 participants, including 434 patients with GU and 347 controls, were recruited for the study. The participants were enrolled according to the inclusion criteria: birthplace in Central Russia and Russian ethnicity (self-reported) [19, 20].

All participants were examined by qualified gastroenterologists. GU and complications (if any) were diagnosed by conventional clinical and endoscopic examinations. The control

group consisted of healthy individuals without any symptoms of gastrointestinal disease [21]. Endoscopy was not performed in healthy individuals because of both ethical reasons and the low probability of finding an active ulcer in patients without the symptoms [22]. Patients and control group volunteers having used NSAIDs, corticosteroids, and aspirin for a long-term treatment were excluded.

The *H. pylori* infection in patients was diagnosed by positive findings on histologic examination of biopsies obtained during endoscopy procedures by a certified pathologist and using the Giemsa stain protocol [23]. Among 434 patients with GU, 196 were *H. pylori*-positive and 238 were *H. pylori*-negative. In controls, the presence of *H. pylori* was determined using a commercial IgG ELISA kit (Plate Helicobacter IgG, Roche). Control group volunteers with the presence of *H. pylori* infection were excluded.

The study protocol was approved by the Medical Institution Ethics Committee of Belgorod State University. All study participants signed informed consent prior to enrolment in the study. The clinical and endoscopic examination of the participants was conducted at the Gastroenterology Division of Belgorod Regional Clinical Hospital.

DNA isolation and genotyping assay

Whole blood samples (5 mL) were collected from all study participants into EDTA-containing tubes and maintained at − 20˚C until processed [24, 25]. Genomic DNA was extracted from the buffy coat by the phenol/chloroform method as described earlier [26].

Ten SNPs of the *MMP* genes (rs1799750 *MMP1*, rs243865 *MMP2*, rs679620 *MMP3*, rs1940475 *MMP8*, rs3918242, rs3918249, rs3787268, rs17576, rs17577, and rs2250889 *MMP9*) were selected for this study according to the criteria [27, 28]: previously reported associations with digestive diseases (gastric and duodenal ulcer, gastric cancer, etc.), regulatory potential, and MAF > 0.05.

All selected SNPs had significant regulatory potential as evidenced by the HaploReg online tools [29] (S1 Table); eight polymorphisms were associated with digestive diseases (gastric and duodenal ulcer, gastric and esophageal cancer, digestive cancers, gastritis) (including two SNPs associated with the peptic ulcer) in previously published candidate gene association studies (S2 Table). Two SNPs (rs3918249 and rs3787268 *MMP9*) did not demonstrate a significant association with digestive diseases but had significant regulatory potential (according to HaploReg).

The genotyping was performed using the MassARRAY® 4 System by Agena Bioscience®. Blind replicates were genotyped to control the quality [30]. Laboratory personnel that conducted genotyping was blinded to patients' information. The repeatability test was performed for 5% of randomly selected samples, yielded 100% reproducibility.

Statistical and functional analysis

The observed allele and genotype frequencies were checked for the correspondence to the Hardy-Weinberg equilibrium using the chi-square test [31]. Associations of the SNPs with GU were analyzed using the logistic regression and assuming dominant, log-additive, and recessive genetic models [32]. The regression analysis was adjusted for covariates: BMI as a quantitative variable, whereas a family history of peptic ulcer, alcohol and tobacco consumption, stress, the presence of cardiovascular and endocrine pathology were applied as qualitative parameters (Table 1). The given sample size (434 patients with GU and 347 controls) was sufficient to detect differences in allelic frequencies between the affected subjects and controls at OR = 1.33–1.82 for the additive model, OR = 1.58–1.86 for the dominant model and OR = 1.61–27.0 for the recessive model (at 80% power, $\alpha = 0.05$ for 2-sided test). Statistical

Table 1. Phenotypic characteristics of the study participants.

Parameters	Control	GU	p
	mean ± SD, % (n)	mean ± SD, % (n)	
N	347	434	-
Age, years (min–max)	48.47±13.69 (22–79)	49.08±11.18 (22–79)	0.36
Gender ratio, f/m	66.28/33.72 (230/117)	68.66/31.34 (298/136)	0.53
BMI, kg/m2	26.83±5.09	27.93±5.02	**0.003**
Age of developing peptic ulcer, years	-	45.47±12.08	-
Family history of peptic ulcer	4.32 (15)	17.05 (74)	**0.0005**
Current smoking	14.99 (52)	25.35 (110)	**0.001**
Alcohol consumption	32.28 (112)	49.31 (214)	**0.0005**
Stress	37.17 (129)	79.26 (344)	**0.0005**
Positivity *H. pylori* test (endoscopic biopsy and histological identification)	-	45.16 (196)	-
Anatomical characteristics of the ulcer			
Location			
Stomach: Body	-	5.07 (22)	-
Pylorus	-	5.53 (24)	-
Antrum	-	89.40 (388)	-
Sizes ulcer (diameter) (cm)	-	0.50±0.36	-
Sizes ulcer: Small (<0.5 cm)	-	64.06 (278)	-
Medium (0.5–1.0 cm)	-	28.11 (124)	-
Large (>1.0 cm)	-	7.83 (32)	-
Associated complications			
Bleeding	-	1.38 (6)	-
Perforation	-	5.99 (26)	-
Stenosis	-	2.30 (10)	-
Malignancy	-	3.23 (14)	-
Somatic pathologies			
Cardiovascular pathology	26.80 (93)	60.83 (264)	**0.0005**
Endocrine pathology	3.17 (11)	7.37 (32)	**0.02**
Kidney pathology	2.59 (9)	4.61 (20)	0.20
Respiratory system pathology	4.32 (15)	5.53 (24)	0.55
Nervous system pathology	7.78 (27)	9.68 (42)	0.42
Musculoskeletal system pathology	6.91 (24)	8.29 (36)	0.56

P values <0.05 are shown in bold.

https://doi.org/10.1371/journal.pone.0257060.t001

power for each SNP was estimated using Quanto 1.2.4 [33]. The haplotype blocks were identified using the «Solid Spine» algorithm (D' > 0.8) as implemented in HaploView v.4.2 [34]. The association analyses and adjustment for multiple comparisons by the adaptive permutation test [35] were conducted using the PLINK v. 2.0 software [36]. The significance value was set at $p_{perm}<0.017$ (after the Bonferroni correction based on the numbers of paired comparisons, n = 3: GU–control, *H. pylori*-positive GU—control, and *H. pylori*-negative GU—control).

The functional importance (missense replacement, eQTLs and sQTLs, regulatory potential) of the genetic variants associated with GU and those linked to them was studied *in silico* [37, 38]. The SIFT online tool [39] was used to identify missense replacements and predict their functional effects. The SNP epigenetic effects were analyzed using RegulomeDB [40] and HaploReg [29]. The data from the GTExportal browser [41] was used to estimate the influence of

Table 2. Associations of the *MMP* gene polymorphisms with GU.

SNP	Gene	MAF	n	Additive model				Dominant model				Recessive model			
				OR	95%CI		P	OR	95%CI		P	OR	95%CI		P
					L95	U95			L95	U95			L95	U95	
rs1940475	*MMP8*	T	776	0.96	0.76	1.22	0.733	0.89	0.61	1.31	0.569	1.00	0.67	1.50	0.989
rs1799750	*MMP1*	2G	751	0.87	0.68	1.11	0.257	0.80	0.55	1.18	0.261	0.85	0.56	1.30	0.457
rs679620	*MMP3*	T	773	1.01	0.79	1.30	0.917	1.06	0.71	1.58	0.793	0.98	0.65	1.47	0.924
rs243865	*MMP2*	T	763	0.99	0.75	1.31	0.931	0.95	0.67	1.36	0.789	1.11	0.57	2.17	0.765
rs3918242	*MMP9*	T	767	0.76	0.54	1.08	0.131	0.76	0.51	1.12	0.165	0.56	0.17	1.82	0.336
rs3918249	*MMP9*	C	767	1.12	0.87	1.44	0.367	1.45	1.00	2.09	0.049	0.79	0.48	1.30	0.363
rs17576	*MMP9*	G	776	1.17	0.91	1.51	0.225	1.27	0.89	1.82	0.194	1.16	0.71	1.90	0.564
rs3787268	*MMP9*	A	775	1.23	0.90	1.67	0.188	1.37	0.96	1.96	0.081	0.76	0.29	1.95	0.564
rs2250889	*MMP9*	G	770	0.89	0.62	1.29	0.533	0.90	0.59	1.37	0.616	0.69	0.20	2.31	0.546
rs17577	*MMP9*	A	760	0.76	0.54	1.07	0.120	0.79	0.54	1.18	0.252	0.33	0.09	1.19	0.090

All results were obtained after adjustment for covariates. OR, odds ratio; 95%CI, 95% confidence interval.

the GU-candidate loci on mRNA levels and splicing QTLs. Likewise, regulatory potential, eQTL and sQTL values of polymorphisms in strong linkage disequilibrium (LD, $r^2 \geq 0.8$) with the GU-associated loci were estimated [42, 43]. The linked SNPs were identified using HaploReg [29].

Results

Baseline and clinical characteristics of the participants are presented in Table 1. The GU patients had higher BMI (p = 0.003), higher percentage of positive family history of peptic ulcer (p = 0.0005), alcohol (p = 0.0005) and tobacco (p = 0.001) consumption, stress (p = 0.0005), the presence of cardiovascular (p = 0.0005) and endocrine (p = 0.02) pathology as compared to the healthy participants (the data are shown in Table 1). Therefore, these parameters were used as confounding factors in the logistic regression analyses.

The summary data about the studied SNPs is given in S3 Table. All SNPs corresponded to the HWE (p>0.005, p_{bonf}>0.05). None of the SNPs was independently associated with GU according to any of the genetic models (Table 2). As to *H. pylori*-positive GU, only loci of the *MMP9* gene manifested association with the disease. Allele C at rs3918249 *MMP9* locus was associated with *H. pylori*-positive GU according to the dominant model (OR = 2.02, 95% CI 1.21–3.37, p_{perm} = 0.008, power—94.68%), and allele A at rs3787268 *MMP9* was associated with *H. pylori*-positive GU according to the additive (OR = 1.60 95% CI 1.09–2.34, p_{perm} = 0.016, power = 89.98%) and dominant (OR = 1.82 95% CI 1.14–2.89, p_{perm} = 0.012, power = 91.14%) genetic models (the data are provided in Table 3). Several haplotypes of the studied SNPs *MMP9* gene were associated with GU (four SNPs within two haplotypes, OR = 1.62–1.65, p_{perm} ≤ 0.006) and *H. pylori*-positive GU (all six SNPs within eight haplotypes, OR = 1.85–2.04, p_{perm} ≤ 0.016) (Table 4, Fig 1). None of the SNPs was associated with *H. pylori*-negative GU either independently or within haplotypes (Table 3).

Functional SNP predictions

Non-synonymous SNPs. Among the six loci of the *MMP9* gene associated with *H. pylori*-positive GU, three SNPs were missense variant: rs17576 (amino acid change Gln279Arg, SIFT score 0.29, SIFT prediction «tolerated»), rs2250889 (Arg574Pro, SIFT score 1.00, SIFT prediction «tolerated») and rs17577 (Arg668Gln, SIFT score 0.02, SIFT prediction «deleterious»).

Table 3. Associations of the *MMP* gene polymorphisms with *H. pylori*-positive and *H. pylori*-negative GU.

SNP	Gene	MAF	n	Additive model				Dominant model				Recessive model			
				OR	95%CI		P	OR	95%CI		P	OR	95%CI		P
					L95	U95			L95	U95			L95	U95	
							H. pylori-positive GU								
rs1940475	MMP8	T	540	0.97	0.71	1.32	0.833	0.86	0.52	1.42	0.556	1.07	0.63	1.81	0.796
rs1799750	MMP1	2G	523	0.81	0.59	1.12	0.212	0.79	0.48	1.30	0.345	0.71	0.40	1.28	0.254
rs679620	MMP3	T	537	0.91	0.65	1.26	0.568	0.96	0.57	1.63	0.887	0.80	0.46	1.39	0.426
rs243865	MMP2	T	533	0.94	0.64	1.37	0.742	0.87	0.54	1.40	0.563	1.15	0.47	2.81	0.752
rs3918242	MMP9	T	533	0.87	0.56	1.36	0.539	0.94	0.57	1.56	0.811	0.28	0.03	2.23	0.228
rs3918249	MMP9	C	533	1.30	0.94	1.80	0.115	**2.02**	**1.21**	**3.37**	**0.007**	0.84	0.44	1.60	0.600
rs17576	MMP9	G	538	1.45	1.04	2.02	0.028	1.76	1.07	2.89	0.026	1.47	0.80	2.71	0.215
rs3787268	MMP9	A	539	**1.60**	**1.09**	**2.34**	**0.016**	**1.82**	**1.14**	**2.89**	**0.012**	1.52	0.55	4.17	0.419
rs2250889	MMP9	G	532	0.97	0.60	1.57	0.910	0.99	0.57	1.73	0.984	0.79	0.16	3.79	0.766
rs17577	MMP9	A	526	0.91	0.59	1.42	0.684	1.03	0.62	1.70	0.921	0.24	0.03	1.89	0.175
							H. pylori-negative GU								
rs1940475	MMP8	T	582	0.97	0.72	1.30	0.825	0.96	0.60	1.54	0.858	0.95	0.58	1.56	0.852
rs1799750	MMP1	2G	567	0.92	0.69	1.23	0.573	0.82	0.52	1.31	0.414	0.98	0.59	1.62	0.923
rs679620	MMP3	T	581	1.09	0.81	1.48	0.559	1.13	0.69	1.85	0.630	1.13	0.69	1.83	0.635
rs243865	MMP2	T	573	1.03	0.73	1.45	0.856	1.02	0.66	1.58	0.923	1.11	0.49	2.51	0.799
rs3918242	MMP9	T	577	0.68	0.44	1.06	0.089	0.62	0.37	1.03	0.064	0.80	0.21	2.99	0.740
rs3918249	MMP9	C	579	0.97	0.71	1.31	0.838	1.01	0.71	1.71	0.671	0.73	0.39	1.37	0.331
rs17576	MMP9	G	584	0.97	0.71	1.32	0.839	0.98	0.64	1.51	0.933	0.91	0.48	1.72	0.775
rs3787268	MMP9	A	581	0.93	0.63	1.37	0.708	1.05	0.68	1.63	0.816	0.18	0.02	1.43	0.106
rs2250889	MMP9	G	580	0.83	0.52	1.31	0.421	0.82	0.49	1.39	0.469	0.63	0.13	3.03	0.566
rs17577	MMP9	A	574	0.64	0.41	1.00	0.050	0.62	0.37	1.03	0.067	0.39	0.09	1.80	0.228

All results were obtained after adjustment for covariates. OR, odds ratio; 95%CI, 95% confidence interval.

https://doi.org/10.1371/journal.pone.0257060.t003

Regulatory effects predictions. According to RegulomeDB and HaploReg, all six SNPs of the *MMP9* gene (rs3918242, rs3918249, rs17576, rs3787268, rs2250889 and rs17577) associated with *H. pylori*-positive GU possess significant regulatory effects. The RegulomeDB suggests

Table 4. Significant associations of the *MMP9* gene haplotypes with GU and *H. pylori*-positive GU.

SNPs	Haplotype	Frequency		OR	$P_{raw\ value}$	P_{perm}
		Cases	Controls			
		GU				
rs3918242-rs3918249-rs17576	CCG	0.2568	0.1846	1.65	0.002	0.004
rs3918242-rs3918249-rs17576-rs3787268	CCGA	0.2306	0.1679	1.62	0.004	0.006
		H. pylori-positive GU				
rs3918242-rs3918249-rs17576	CCG	0.2827	0.1841	1.92	0.001	0.005
rs3918242-rs3918249-rs17576-rs3787268	CCGA	0.2650	0.1639	2.04	0.0007	0.002
rs3918242-rs3918249-rs17576-rs3787268-rs2250889	CCGAC	0.2563	0.1581	2.04	0.001	0.004
rs3918242-rs3918249-rs17576-rs3787268-rs2250889-rs17577	CCGACG	0.2511	0.1652	1.90	0.002	0.016
rs3918249-rs17576-rs3787268	CGA	0.2654	0.1721	1.88	0.003	0.011
rs3918249-rs17576-rs3787268-rs2250889-rs17577	CGACG	0.2523	0.1634	1.96	0.002	0.011
rs17576-rs3787268	GA	0.2733	0.1802	1.85	0.003	0.010
rs17576-rs3787268-rs2250889-rs17577	GACG	0.2538	0.1607	2.00	0.001	0.010

Note: All results were obtained after adjustment for covariates; OR, odds ratio; P, significance level.
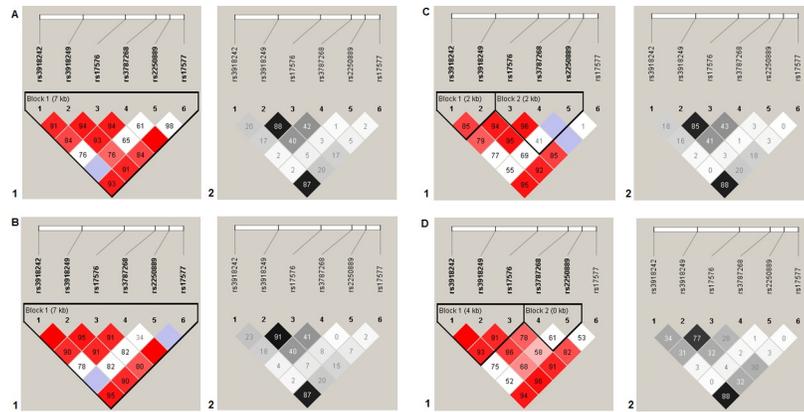
https://doi.org/10.1371/journal.pone.0257060.t004

Fig 1. Linkage disequilibrium (LD) between SNPs rs3918242, rs3918249, rs17576, rs3787268, rs2250889 and rs17577 of the *MMP9* gene in GU patients. A, summary; B, *H. pylori*-positive GU patients; C, *H. pylori*-negative GU patients; D, control group. LD values are given as Lewontin's standardized coefficient D′ (Figure sections 1) and the square of the Pearson's correlation coefficient (r^2) (Figure sections 2) between SNPs.

https://doi.org/10.1371/journal.pone.0257060.g001

the most significant regulatory potential for rs17577 (rank - 2b, score—0.79). The other five *H. pylori*-positive GU-associated SNPs (rs3918242, rs3918249, rs17576, rs3787268, rs2250889) have RegulomeDB rank = 4–5 and RegulomeDB score = 0.59–0.61. According to the Hap-loReg database, the above six polymorphisms were located in DNase I hypersensitive sites, five SNPs–in the specific region of DNA binding with modified histone marking promoters (rs3918249, rs17576, rs3787268, rs2250889 and rs17577) and enhancers (rs3918242, rs3918249, rs17576, rs3787268 and rs17577) in various tissues, and in the sixteen motifs to the transcription factors (TFs), three SNPs (rs17576, rs2250889 and rs17577)—in evolutionarily conserved DNA segments, and two SNPs (rs2250889 and rs17577)—in the protein-bound site (S1 Table). Herewith, alleles of the *MMP9* gene loci associated with increased risk for *H. pylori*-positive GU (Table 4) increases affinity to twelve TFs (Ahr:Arnt, HIF1, Myc, Hmx, Hoxb8, HDAC2, Mef2, Pou1f1, Sox, Zfp105, p300, NRSF) and decreases affinity to the four TFs (E2F, Arid3a, Pax-5, Pax-4) (S4 Table).

In addition to the six *H. pylori*-positive GU-related SNPs, regulatory significance was esti-mated for 65 loci linked to them (the data are provided in S5 Table). Seventeen SNPs have been positioned in evolutionarily conserved DNA regions. Nine SNPs (including four synony-mous and five missense replacements) were located in protein-coding regions (exons) of the *MMP9* gene, 26 in introns, and 30 in intergenic areas. All 65 SNPs linked to the *H. pylori*-posi-tive GU-associated SNPs had a significant regulatory potential; several polymorphisms mani-fested epigenetic effects (S5 Table). For example, rs6073989 (linked to rs3787268, $r^2 = 0.95$) is positioned in the specific area of DNA binding with modified histone marking promoters (H3K4me3, H3K9ac) and enhancers (H3K4me1, H3K27ac) in more than ten tissues/organs, the DNAase I hypersensitive segments in sixteen tissues/organs, and a sites of five regulatory DNA motifs (CAC-binding-protein, EWSR1-FLI1, PRDM1, SP1, TATA). The SNP rs6073991, which also was in linkage disequilibrium with rs3787268 ($r^2 = 0.95$), was located in the hypersensitive region to DNAase-I in 49 (!) tissues/organs, in the protein-bound region (with this DNA region interact three regulatory proteins—NRSF, ZNF143, BCL3), and a puta-tive transcription factor binding sites (SZF1-1, T3R) (S5 Table). Importantly, the epigenetic effects of the *H. pylori*-positive GU-associated SNPs and 65 polymorphisms linked to them of the *MMP9* gene were reported for the target organs of GU, adult stomach mucosa and smooth muscle, fetal stomach.

Expression quantitative trait loci (eQTLs). Referring to the data of the GTExportal resource, six *H. pylori*-positive GU-associated polymorphisms *MMP9* gene and 61 SNPs linked to them had the eQTL significance (*cis*-eQTL and *trans*-eQTL) and correlated with mRNA levels of 17 genes in more than 25 various tissues and organs (*NEURL2*, *SLC12A5*, *CD40*, *MMP9*, *RP3-337O18.9*, *NTTIP1*, *PCIF1*, *SPATA25*, *RP11-465L10.10*, *ZSWIM1*, *RPL13P2*, *SNX21*, *WFDC10B*, *PLTP*, *SYS1*, *WFDC3*, *ZNF335*) (S6 and S7 Tables). For example, rs3787268 and rs3918249 (individually associated with *H. pylori*-positive GU) may affect the expression genes (*RP3-337O18.9*, *PLTP*, *NEURL2*) in the organs of the digestive system (esophagus, various sections of the colon) as well as other tissues/organs related to the development of GU: brain (frontal cortex and pituitary (*PLTP*)), adipose tissue (visceral and subcutaneous) (*NEURL2*, *SPATA25*, *PLTP*, *SLC12A5*, *CD40*, *ZSWIM1*, *RP3-337O18.9)*, whole blood (*ZNF335*), thyroid (*PLTP*, *NEURL2*), adrenal gland (*PLTP*, *PCIF1*, *SLC12A5*, *RP11-465L10.10*), etc. Importantly, the risk alleles of the analyzed loci (e.g., A rs3787268 and C rs3918249 C) are usually associated with the lower expression of the genes (S6 Table).

Splicing quantitative trait loci (sQTLs). Analysis of the GTExportal data suggested that the six GU-associated loci of *MMP9* had significant sQTL values and might influence alternative splicing of five genes (*SLC12A5*, *ACOT8*, *CD40*, *SNX21*, *PLTP*) in various tissues and organs (the results are shown in S8 Table). These polymorphisms manifested strong linkage to 61 SNPs, which affect splicing QTL of six genes (*CD40*, *SLC12A5*, *ACOT8*, *SNX21*, *PLTP*, *SLC35C2*) in more than 15 organs and tissues (S9 Table). Importantly, the independently associated with *H. pylori*-positive GU loci rs3787268 and rs3918249 correlate with splicing QTLs of the *PLTP* gene in subcutaneous adipose tissue and *SLC12A5* gene in the various parts of the brain (e.g., cortex and substantia nigra, pituitary), which are known to play a role in the pathogenesis of GU. Interestingly, the GU risk allele, C rs3918249, correlates with the elevated level of in the sQTL of the *SLC12A5* gene (β>0) in the substantia nigra and pituitary and with the lower level of the sQTL of the *SLC12A5* gene (β<0) in the brain cortex. The GU risk allele A rs3787268 is associated with the higher level of the sQTL of the *PLTP* gene in the subcutaneous adipose tissue (S8 Table). Besides, rs3787268 and rs3918249 each are in strong LD with eleven sQTL SNPs (S9 Table).

Discussion

In the present study, we report for the first time the association of the SNPs rs3918249 and rs3787268 of *MMP9* with *H. pylori*-positive GU but not with *H. pylori*-negative GU in the Caucasian population of Central Russia. Alleles C of rs3918249 *MMP9* (OR = 2.02) and A of rs3787268 *MMP9* (OR = 1.60–1.82), and eight haplotypes of the six studied SNPs of the *MMP9* gene (OR = 1.85–2.04) increased risk for *H. pylori*-positive GU. None of the *MMP* gene SNPs was independently associated with GU. Also, two haplotypes of the *MMP9* gene (contributed by four SNPs, rs3918242, rs3918249, rs17576, and rs3787268) increased risk for GU (OR = 1.62–1.65). Six loci of the *MMP9* gene, which are associated with *H. pylori*-positive GU, and 65 SNPs linked to them manifest significant epigenetic effects, have pronounced eQTL (17 genes) and sQTL (six genes) values in the organs of the digestive system and the other tissues/organs, which have been suggested to contribute to GU.

Only two genome-wide association studies (GWAS) of the peptic ulcer disease (PUD) have been conducted so far [44, 45]. One of them reported only two SNPs (rs2294008 *PSCA* and rs505922 *ABO*) associated with duodenal ulcer in Japanese [44]; another determined eight PUD-associated loci in the *MUC1*, *MUC6*, *FUT2*, *PSCA*, *ABO*, *CDX2*, *GAST* and *CCKBR* genes, including the two reported for the Japanese cohort [45]. While the estimated heritability for PUD is about 28% [45], only 6% of the estimated variance in the trait is attributed to

genome-wide common SNPs (i.e., SNP-based heritability) [45] that raises a problem of so-called "missing heritability". This problem may be addressed by studying associations of candidate genes for peptic ulcer. In these terms, the *MMPs* genes are very good candidates, as they are related to both the pathogenesis of *H. pylori*-associated gastric ulcer and the inflammatory response of the mucosa [13].

So far, only one study [13] reported a significant association of an *MMP9* gene variant with *H. pylori*-positive GU. The authors analyzed 20 SNPs of the *MMP1*, *3*, *7*, and *9* genes in the sample of 599 *H. pylori*-infected German patients and determined that two SNPs were associated with the disease: the rs17576 polymorphism of the *MMP9* gene and another variant in the promoter region of the *MMP7* gene. Interestingly, Hellmig S. et al. determined allele A of rs17576 as a risk factor for the disease [13], while our results suggested allele variant G of the same locus (within the significantly associated haplotypes of the *MMP9* gene) as the risk factor for the Caucasian population of Central Russia. Yeh et al. [18] did not find any significant association of the above SNP with the disease in a Taiwanese population. Thus, our study is the first that reports the association of rs3918242, rs3918249, rs3787268, rs2250889, rs17577 of the *MMP9* gene with *H. pylori*-positive GU.

There are quite a few studies, which analyzed the association of *MMP* gene polymorphisms with gastric cancer (S2 Table) and reported such an association for the *MMP9* gene SNPs included in our study (rs3918242, rs17576, rs17577). Notably, allele C of rs3918242, a risk factor for *H. pylori*-positive GU in Caucasians from Central Russia in the present study, also increased the risk for gastric cancer [46, 47] and esophageal cancer [48]. It is thought that *H. pylori*-associated GU is positively linked to stomach cancer due to the damage of gastric mucosa [49] and the *MMP9* gene may be one of the candidate genes involved in both *H. pylori*-associated GU and gastric cancer [50].

The *MMP9* gene is located on chromosome 20q11.1–13.1. The encoded protein (type IV collagenase, also known as gelatinase B) can degrade various collagen (collagen types IV, V, VII, X, XIV, gelatin) and non-collagen substrates (elastin, aggrecan, fibronectin, nidogen, laminin, etc.) of the extracellular matrix (ECM) [51]. ECM of the gastric mucosa contains a significant proportion of collagen, elastin, fibronectin, laminin, hyaluronic acid, and proteoglycan, and their degradation by MMPs is important for the stability of the cellular microenvironment [13]. MMP9 is a key enzyme implicated in gastric ulcer [52, 53]. ECM degradation by MMPs is apparently a key factor contributing to gastric mucosal damage [6]. The significant elevation of the MMP9 level in gastric ulcer tissues suggested that the enzyme may regulate mucosa lesions in GU by degrading collagens and creating lesions [50]. Also, this enzyme is important in the early phase of chronic GU [12].

The present study reports association of the *MMP9* gene polymorphisms with *H. pylori*-positive GU but not with *H. pylori*-negative GU. The available literature also suggests that *MMP9* gene polymorphisms apparently contribute to a genetic risk profile to develop GU in chronic *H. pylori* infection [13]. There is evidence that MMPs can be induced by both *H. pylori* bacterial products and proinflammatory cytokines [54]. MMPs have elevated expression in gastric epithelial cells infected with *H. pylori* that might contribute to the GU pathogenesis. Li et al. [17] analyzed samples of gastric mucosa from the antrum and ulcer site and found that the higher MMP9 expression was associated with the *H. pylori* infection and correlated with the level of inflammation determined histologically at the border of the ulcer. Several studies demonstrated a correlation between the elevated serum levels of MMP9 and higher MMP9 activity in antral mucosa of *H. pylori*-infected patients with gastritis [55, 56]. Antral mucosa of *H. pylori*-infected subjects demonstrates a 19-fold higher MMP9 protein activity than that of uninfected individuals [55] as *H. pylori* induces NF-kappaB activation through the intracellular signaling pathway resulting in transcription of the *MMP9* gene [15]. Notably, the *H. pylori-*

induced *MMP9* up-regulation can be reversed after successful pathogen eradication. If the eradication failed, no difference in the MMP9 protein expression was detected in epithelial cells and fibroblasts before and after the treatment [57]. *H. pylori* plays an important role not only in gastroduodenal diseases (chronic gastritis, peptic ulcer, gastric cancer, etc.) but also in various extragastric pathologies (cardiovascular, endocrine (insulin resistance, diabetes mellitus), etc.) [58]. Indeed, higher BMI, the higher prevalence of cardiovascular and endocrine pathologies, among GU patients (including 45.16% of *H. pylori*-positive) as compared to the controls (*H. pylori*-negative) was documented in the present study (Table 1). Importantly, polymorphisms of the *MMP* genes (including *MMP9*) were suggested to determine the susceptibility to cardiovascular diseases and their complications in various populations [59–61], including Caucasians of Central Russia [42, 62, 63]. Wu et al. [45] documented significant positive SNP-based genetic correlation (r_g) between PUD and BMI, body fat-related traits, and coronary artery disease. The *in silico* analysis in our study also identified significant expression and splicing QTLs among the *H. pylori*-positive GU-associated SNPs of the *MMP9* gene and their proxies affecting several genes (e.g., *PLTP*, *CD40*, *SLC12A5*, *NEURL2*, *SPATA25*, *ZSWIM1*, *RP3-337O18.9*) in the adipose tissue (visceral and subcutaneous).

The available literature data suggests that the above genes are involved in biological pathways contributing to pathophysiology of GU. For example, the *PLTP* gene encodes one of two phospholipid transfer proteins found in human blood plasma. This protein transfers phospholipids and cholesterol between different classes of lipoproteins and was implicated in many disorders, including hyperlipidemia, obesity, metabolic syndrome, type II diabetes, and others [64]. There is evidence that PLTP may play important roles in the nervous system through participation in signal transduction pathways [65], control of vitamin E content [66], and maintenance of blood-brain barrier integrity [67]. Another example is the *CD40* gene. It is a member of the tumor necrosis factor receptor superfamily and encodes a protein playing a key role in a broad range of immune and inflammatory responses including T cell activation, immunoglobulin isotype switching and cytokine production [68]. *CD40* has been implicated in various disorders, such as atherosclerosis, cardiovascular, metabolic (arterial hypertension, diabetes mellitus, etc.), and the others [68, 69]. Importantly, the *CD40* DNA methylation levels [70] and CD40 expression (protein and mRNA) [71] were associated with gastric cancer. The *ACOT8* gene encodes a peroxisomal thioesterase (acyl-CoA thioesterase 8) involved in the oxidation of fatty acids [72]. This enzyme is localized ubiquitously throughout all cellular compartments and is among the key enzymes of lipid metabolism [72].

Due to their pleiotropism, polymorphisms of the *MMP9* genes play a key role in multiple biological pathways and therefore have been implicated not only in peptic ulcer [13, 73, present study], but also in cardiovascular diseases [60, 61] as well as a broad range of other disorders involving processes of synthesis and degradation of extracellular matrix: various cancers, including digestive [46–48, 74–76], glaucoma [27, 77, 78], and others [79–82].

One limitation of this study should be acknowledged though. Like other association studies, our study did not utilize any experimental procedures to test the predictions about the possible functional significance of the *H. pylori*-positive GU-associated SNPs of the *MMP9* gene, because "wet" experiments were beyond the study scope. The predictions were made based solely on the *in silico* analysis of the available functional genomics databases (HaploReg [29], GTExportal browser [41], SIFT online tool [39], RegulomeDB [40]), which include data of large-scale studies in this area (Genotype-Tissue Expression (GTEx) project, Roadmap Epigenomics and ENCODE projects, etc.).

Conclusions

The *MMP9* gene polymorphisms were associated with *H. pylori*-positive GU but not with *H. pylori*-negative GU in the Caucasian population of Central Russia. Alleles C rs3918249 *MMP9* (OR = 2.02) and A rs3787268 *MMP9* (OR = 1.60–1.82), and eight haplotypes of the all studied six SNPs *MMP9* gene (OR = 1.85–2.04) increased risk for *H. pylori*-positive GU. None of the *MMPs* genes SNPs was independently associated with GU. Also, two haplotypes *MMP9* gene (including the following SNPs, rs3918242, rs3918249, rs17576, and rs3787268) increased risk for GU (OR = 1.62–1.65). Six loci of the *MMP9* gene associated with *H. pylori*-positive GU and 65 SNPs linked to them manifest significant epigenetic effects, have noticeable eQTL (17 genes) and sQTL (6 genes) values.

Supporting information

S1 Table. The regulatory potential of the studied SNPs.
(XLS)

S2 Table. The literature data about associations of the studied polymorphisms of the MMP **genes with some digestive diseases.**
(DOC)

S3 Table. The allele and genotype frequencies of the studied MMP gene SNPs in the GU patients and control group.
(DOC)

S4 Table. Effect of the MMP-9 gene polymorphisms on affinity of the DNA regulatory motifs.
(DOCX)

S5 Table. Regulatory effects of the six *H. pylori*-positive GU-associated SNPs of the MMP9 gene and SNPs in high LD (r2≥0.80).
(XLS)

S6 Table. eQTL values of the *H. pylori*-positive GU-associated SNPs MMP-9 gene.
(XLS)

S7 Table. Effect of SNPs in high LD (r2≥0.80) with the *H. pylori*-positive GU-associated polymorphisms MMP-9 gene on gene expression level.
(XLS)

S8 Table. sQTL values of the *H. pylori*-positive GU-associated SNPs MMP-9 gene.
(XLS)

S9 Table. Effect of SNPs in high LD (r2≥0.80) with the *H. pylori*-positive GU-associated polymorphisms MMP-9 gene on alternative splicing level.
(XLS)

S1 References. References to S2 Table.
(DOC)

Author Contributions

Conceptualization: Volodymyr Dvornyk, Mikhail Churnosov.

Data curation: Oksana Minyaylo.

Formal analysis: Irina Ponomarenko, Evgeny Reshetnikov.

Funding acquisition: Mikhail Churnosov.

Methodology: Volodymyr Dvornyk, Oksana Minyaylo, Evgeny Reshetnikov, Mikhail Churnosov.

Project administration: Volodymyr Dvornyk, Mikhail Churnosov.

Supervision: Volodymyr Dvornyk, Mikhail Churnosov.

Validation: Evgeny Reshetnikov.

Writing – original draft: Irina Ponomarenko, Oksana Minyaylo.

Writing – review & editing: Volodymyr Dvornyk, Irina Ponomarenko, Oksana Minyaylo, Evgeny Reshetnikov, Mikhail Churnosov.

References

1. Fashner J and Gitu AC. Diagnosis and Treatment of Peptic Ulcer Disease and H. pylori Infection. Am Fam Physician 2015; 91: 236–242. PMID: 25955624

2. Kang JH, Lim YJ, Kang JH, Yang JN, Shin SM, Choi JH, et al. Prevalence of Precancerous Conditions and Gastric Cancer Based upon the National Cancer Screening Program in Korea for 7 Years, Single Center Experience. Gastroenterol Res Pract. 2015; 2015: 571965. https://doi.org/10.1155/2015/571965 PMID: 25642244

3. Carli DM, Pires RC, Rohde SL, Kavalco CM, Fagundes RB. Peptic ulcer frequency differences related to H. Pylori or aines. Arq Gastroenterol. 2015; 52: 46–49. https://doi.org/10.1590/S0004-28032015000100010 PMID: 26017082

4. Yazbek PB, Trindade AB, Chin CM and Dos Santos JL. Challenges to the Treatment and New Perspectives for the Eradication of Helicobacter pylori. Dig Dis Sci. 2015; 60: 2901–2912. https://doi.org/10.1007/s10620-015-3712-y PMID: 25999247

5. Tarnawski AS, Ahluwalia A. Molecular mechanisms of epithelial regeneration and neovascularization during healing of gastric and esophageal ulcers. Curr Med Chem. 2012; 19: 16–27. https://doi.org/10.2174/092986712803414088 PMID: 22300072

6. Ganguly K, Kundu P, Banerjee A, Reiter RJ, Swarnakar S. Hydrogen peroxide-mediated downregulation of matrix metalloprotease-2 in indomethacin-induced acute gastric ulceration is blocked by melatonin and other antioxidants. Free Radic Biol Med. 2006; 41: 911–925. https://doi.org/10.1016/j.freeradbiomed.2006.04.022 PMID: 16934674

7. Shahin M, Konturek JW, Pohle T, Schuppan D, Herbst H, Domschke W. Remodeling of extracellular matrix in gastric ulceration. Microsc Res Tech. 2001; 53:396–408. https://doi.org/10.1002/jemt.1108 PMID: 11525257

8. Chakraborty S, Stalin S, Das N, Choudhury ST, Ghosh S, Swarnakar S. The use of nano-quercetin to arrest mitochondrial damage and MMP-9 upregulation during prevention of gastric inflammation induced by ethanol in rat. Biomaterials. 2012; 33:2991–3001. https://doi.org/10.1016/j.biomaterials.2011.12.037 PMID: 22257724

9. Kim SJ, Park YS, Paik HD, Chang HI. Effect of anthocyanins on expression of matrix metalloproteinase-2 in naproxen-induced gastric ulcers. Br J Nutr. 2011; 106: 1792–1801. https://doi.org/10.1017/S000711451100242X PMID: 21733337

10. Singh LP, Mishra A, Saha D, Swarnakar S. Doxycycline blocks gastric ulcer by regulating matrix metalloproteinase-2 activity and oxidative stress. World J Gastroenterol. 2011; 17: 3310–3321. https://doi.org/10.3748/wjg.v17.i28.3310 PMID: 21876619

11. Pradeepkumar Singh L, Vivek Sharma A, Swarnakar S. Upregulation of collagenase-1 and -3 in indomethacin-induced gastric ulcer in diabetic rats: role of melatonin. J Pineal Res. 2011; 51: 61–74. https://doi.org/10.1111/j.1600-079X.2010.00845.x PMID: 21342246

12. Kim SJ, Lee HJ, Kim BS, Lee D, Lee SJ, Yoo SH, et al. Antiulcer activity of anthocyanins from Rubus coreanus via association with regulation of the activity of matrix metalloproteinase-2. J Agric Food Chem. 2011; 59: 11786–11793. https://doi.org/10.1021/jf104192a PMID: 21942741

13. Hellmig S, Ott S, Rosenstiel P, Robert Fölsch U, Hampe J, Schreiber S. Genetic variants in matrix metalloproteinase genes are associated with development of gastric ulcer in H. Pylori infection. Am J Gastroenterol. 2006; 101: 29–35. https://doi.org/10.1111/j.1572-0241.2005.00348.x PMID: 16405530

14. Yeh YC, Sheu BS, Cheng HC, Wang YL, Yang HB, Wu JJ. Elevated serum matrix metalloproteinase-3 and -7 in H. pylori-related gastric cancer can be biomarkers correlating with a poor survival. Dig Dis Sci. 2010; 55(6):1649–1657. https://doi.org/10.1007/s10620-009-0926-x PMID: 19690958

15. Mori N, Sato H, Hayashibara T, Senba M, Geleziunas R, Wada A, et al. Helicobacter pylori induces matrix metalloproteinase-9 through activation of nuclear factor kappaB. Gastroenterology. 2003; 124 (4):983–992. https://doi.org/10.1053/gast.2003.50152 PMID: 12671895

16. Crawford HC, Krishna US, Israel DA, Matrisian LM, Washington MK, Peek RM Jr. Helicobacter pylori strain-selective induction of matrix metalloproteinase-7 in vitro and within gastric mucosa. Gastroenterology. 2003; 125(4):1125–1136. https://doi.org/10.1016/s0016-5085(03)01206-x PMID: 14517796

17. Li SL, Zhao JR, Ren XY, Xie JP, Ma QZ, Rong QH. Increased expression of matrix metalloproteinase-9 associated with gastric ulcer recurrence. World J Gastroenterol. 2013; 19(28):4590–4595. https://doi.org/10.3748/wjg.v19.i28.4590 PMID: 23901237

18. Yeh YC, Cheng HC, Chang WL, Yang HB, Sheu BS. Matrix metalloproteinase-3 promoter polymorphisms but not dupA-H. pylori correlate to duodenal ulcers in H. pylori-infected females. BMC Microbiol. 2010; 10:218. https://doi.org/10.1186/1471-2180-10-218 PMID: 20707923

19. Litovkina O, Nekipelova E, Dvornyk V, Polonikov A, Efremova O, Zhernakova N, et al. Genes involved in the regulation of vascular homeostasis determine renal survival rate in patients with chronic glomerulonephritis. Gene. 2014; 546(1):112–6. https://doi.org/10.1016/j.gene.2014.04.020 PMID: 24727057

20. Reshetnikov EA, Akulova LY, Dobrodomova IS, Dvornyk VY, Polonikov AV, Churnosov MI. The insertion-deletion polymorphism of the ACE gene is associated with increased blood pressure in women at the end of pregnancy. J Renin Angiotensin Aldosterone Syst. 2015; 16(3):623–632. https://doi.org/10.1177/1470320313501217 PMID: 24150610

21. Minyaylo ON. Allele distribution and haploblock structure of matrix metalloproteinase gene polymorphism in patients with H. pylori-negative gastric ulcer and duodenal ulcer. Research Results in Biomedicine. 2020; 6(4):488–502. Russian. https://doi.org/10.18413/2658-6533-2020-6-4-0-5

22. García-González MA, Lanas A, Savelkoul PH, Santolaria S, Benito R, Crusius JB, et al. Association of interleukin 1 gene family polymorphisms with duodenal ulcer disease. Clin Exp Immunol. 2003; 134:525–531. https://doi.org/10.1111/j.1365-2249.2003.02325.x PMID: 14632761

23. Lee JY, Kim N. Diagnosis of Helicobacter pylori by invasive test: histology. Ann Transl Med. 2015; 3 (1):10. https://doi.org/10.3978/j.issn.2305-5839.2014.11.03 PMID: 25705642

24. Eliseeva N, Ponomarenko I, Reshetnikov E, Dvornyk V, Churnosov M. The haplotype of the CDKN2B-AS1 gene is associated with primary open-angle glaucoma and pseudoexfoliation glaucoma in the Caucasian population of Central Russia. Ophthalmic Genetics. 2021. https://doi.org/10.1080/13816810.2021.1955275 PMID: 34387529

25. Reshetnikov E, Zarudskaya O, Polonikov A, Bushueva O, Orlova V, Krikun E, et al. Genetic markers for inherited thrombophilia are associated with fetal growth retardation in the population of Central Russia. J Obstet Gynaecol Res. 2017; 43(7):1139–1144. https://doi.org/10.1111/jog.13329 PMID: 28544373

26. Eliseeva N, Ponomarenko I, Reshetnikov E, Dvornyk V, Churnosov M. LOXL1 gene polymorphism candidates for exfoliation glaucoma are also associated with a risk for primary open-angle glaucoma in a Caucasian population from central Russia. Mol Vis. 2021; 27:262–269. PMID: 34012228

27. Starikova D, Ponomarenko I, Reshetnikov E, Dvornyk V, Churnosov M. Novel data about association of the functionally significant polymorphisms of the MMP-9 gene with exfoliation glaucoma in the Caucasian population of Central Russia. 2021; 64(3):458–464. https://doi.org/10.1159/000512507 PMID: 33099542

28. Ponomarenko I, Reshetnikov E, Polonikov A, Verzilina I, Sorokina I, Elgaeva E, et al. Candidate genes for age at menarche are associated with endometriosis. Reprod Biomed Online. 2020; 41(5):943–956. https://doi.org/10.1016/j.rbmo.2020.04.016 PMID: 33051137

29. Ward LD, Kellis M. HaploReg v4: systematic mining of putative causal variants, cell types, regulators and target genes for human complex traits and disease. Nucleic Acids Res. 2016;D1, D877-D881. https://doi.org/10.1093/nar/gkv1340 PMID: 26657631

30. Golovchenko O, Abramova M, Ponomarenko I, Reshetnikov E, Aristova I, Polonikov A, et al. Functionally significant polymorphisms of ESR1and PGR and risk of intrauterine growth restriction in population of Central Russia. Eur J Obstet Gynecol Reprod Biol. 2020; 253:52–57. https://doi.org/10.1016/j.ejogrb.2020.07.045 PMID: 32777541

31. Tikunova E, Ovtcharova V, Reshetnikov E, Dvornyk V, Polonikov A, Bushueva O, et al. Genes of tumor necrosis factors and their receptors and the primary open angle glaucoma in the population of Central Russia. Int J Ophthalmol. 2017; 10:1490–1494. https://doi.org/10.18240/ijo.2017.10.02 PMID: 29062765

32. Ponomarenko I, Reshetnikov E, Polonikov A, Sorokina I, Yermachenko A, Dvornyk V, et al. Candidate genes for age at menarche are associated with endometrial hyperplasia. Gene. 2020; 757:144933. https://doi.org/10.1016/j.gene.2020.144933 PMID: 32640302

33. Gauderman WJ. Sample size requirements for matched case-control studies of gene-environment interaction. Stat Med. 2002; 21(1):35–50. https://doi.org/10.1002/sim.973 PMID: 11782049

34. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21(2):263–5. https://doi.org/10.1093/bioinformatics/bth457 PMID: 15297300

35. Che R, Jack JR, Motsinger-Reif AA, Brown CC. An adaptive permutation approach for genome-wide association study: evaluation and recommendations for use. BioData Min 2014; 7:9. https://doi.org/10.1186/1756-0381-7-9 PMID: 24976866

36. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81(3):559–575. https://doi.org/10.1086/519795 PMID: 17701901

37. Ponomarenko I, Reshetnikov E, Polonikov A, Verzilina I, Sorokina I, Yermachenko A, et al. Candidate genes for age at menarche are associated with uterine leiomyoma. Front Genet. 2021; 11:512940. https://doi.org/10.3389/fgene.2020.512940 PMID: 33552117

38. Polonikov AV, Klyosova EYu, Azarova IE. Bioinformatic tools and internet resources for functional annotation of polymorphic loci detected by genome wide association studies of multifactorial diseases (review). Research Results in Biomedicine. 2021; 7(1):15–31. Russian. https://doi.org/10.18413/2658-6533-2020-7-1-0-2

39. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat. Protoc. 2009; 7:1073–1081. https://doi.org/10.1038/nprot.2009.86 PMID: 19561590

40. Dong S, Boyle AP. Predicting functional variants in enhancer and promoter elements using RegulomeDB. Hum Mutat. 2019; 40(9):1292–1298. https://doi.org/10.1002/humu.23791 PMID: 31228310

41. The GTEx Consortium. Genetic effects on gene expression across human tissues. Nature. 2017; 550:204–213. https://doi.org/10.1038/nature24277 PMID: 29022597

42. Moskalenko M, Ponomarenko I, Reshetnikov E, Dvornyk V, Churnosov M. Polymorphisms of the matrix metalloproteinase genes are associated with essential hypertension in a Caucasian population of Central Russia. Sci Rep. 2021; 11(1):5224. https://doi.org/10.1038/s41598-021-84645-4 PMID: 33664351

43. Ponomarenko I, Reshetnikov E, Altuchova O, Polonikov A, Sorokina I, Yermachenko A, et al. Association of genetic polymorphisms with age at menarche in Russian women. Gene. 2019; 686:228–236. https://doi.org/10.1016/j.gene.2018.11.042 PMID: 30453067

44. Tanikawa C, Urabe Y, Matsuo K, Kubo M, Takahashi A, Ito H, et al. A genome-wide association study identifies two susceptibility loci for duodenal ulcer in the Japanese population. Nat Genet. 2012; 44 (4):430–4, S1-2. https://doi.org/10.1038/ng.1109 PMID: 22387998

45. Wu Y, Murray GK, Byrne EM, Sidorenko J, Visscher PM, Wray NR. GWAS of peptic ulcer disease implicates Helicobacter pylori infection, other gastrointestinal disorders and depression. Nat Commun. 2021; 12(1):1146. https://doi.org/10.1038/s41467-021-21280-7 PMID: 33608531

46. Okada R, Naito M, Hattori Y, Seiki T, Wakai K, Nanri H, et al. Matrix metalloproteinase 9 gene polymorphisms are associated with a multiple family history of gastric cancer. Gastric Cancer. 2017; 20(2):246–253. https://doi.org/10.1007/s10120-016-0608-2 PMID: 27053167

47. Peng Q, Xu Y. Association between promoter polymorphisms of matrix metalloproteinase-1 and risk of gastric cancer. Onco Targets Ther. 2015; 8:2519–2526. https://doi.org/10.2147/OTT.S83004 PMID: 26392779

48. Zhang L, Xi RX, Zhang XZ. Matrix metalloproteinase variants associated with risk and clinical outcome of esophageal cancer. Genet Mol Res. 2015; 14(2):4616–4624. https://doi.org/10.4238/2015.May.4.20 PMID: 25966235

49. Shukla SK, Khatoon J, Prasad KN, Rai RP, Singh AK, Kumar S, et al. Transforming growth factor beta 1 (TGFbeta1) modulates Epstein-Barr virus reactivation in absence of Helicobacter pylori infection in patients with gastric cancer. Cytokine 2015; 77: 176–179. https://doi.org/10.1016/j.cyto.2015.07.023 PMID: 26239415

50. Xu Li., Wang L., Li G., Zheng X., Duan C. Expression of miR-204 and MMP-9 in Helicobacter pylori-associated gastric ulcer. Int J Clin Exp Med. 2016; 9(5):7928–7936.

51. Cui N, Hu M, Khalil RA. Biochemical and Biological Attributes of Matrix Metalloproteinases. Prog Mol Biol Transl Sci. 2017; 147:1-73. https://doi.org/10.1016/bs.pmbts.2017.02.005 PMID: 28413025

52. Swarnakar S, Ganguly K, Kundu P, Banerjee A, Maity P, Sharma AV. Curcumin regulates expression and activity of matrix metalloproteinases 9 and 2 during prevention and healing of indomethacin-

induced gastric ulcer. J Biol Chem. 2005; 280:9409–9415. https://doi.org/10.1074/jbc.M413398200 PMID: 15615723

53. Kundu P, Mukhopadhyay AK, Patra R, Banerjee A, Berg DE, Swarnakar S. Cag pathogenicity island-independent up-regulation of matrix metalloproteinases-9 and -2 secretion and expression in mice by Helicobacter pylori infection. J Biol Chem. 2006; 281:34651–34662. https://doi.org/10.1074/jbc.M604574200 PMID: 16966323

54. Bergin PJ, Raghavan S, Svensson H, Starckx S, Van Aelst I, Gjertsson I, et al. Gastric gelatinase B/matrix metalloproteinase-9 is rapidly increased in Helicobacter felis-induced gastritis. FEMS Immunol Med Microbiol. 2008; 52(1):88–98. https://doi.org/10.1111/j.1574-695X.2007.00349.x PMID: 17995959

55. Bergin PJ, Anders E, Sicheng W, Erik J, Jennie A, Hans L, et al. Increased production of matrix metallo-proteinases in Helicobacter pylori-associated human gastritis. Helicobacter. 2004; 9(3):201–210. https://doi.org/10.1111/j.1083-4389.2004.00232.x PMID: 15165255

56. Rautelin HI, Oksanen AM, Veijola LI, Sipponen PI, Tervahartiala TI, Sorsa TA, et al. Enhanced systemic matrix metalloproteinase response in Helicobacter pylori gastritis. Ann Med. 2009; 41:208–215. https://doi.org/10.1080/07853890802482452 PMID: 18979291

57. Danese S, Papa A, Gasbarrini A, Ricci R, Maggiano N. Helicobacter pylori eradication down-regulates matrix metalloproteinase-9 expression in chronic gastritis and gastric ulcer. Gastroenterology. 2004; 126(1):369–71. https://doi.org/10.1053/j.gastro.2003.06.007 PMID: 14753215

58. Gravina AG, Zagari RM, De Musis C, Romano L, Loguercio C, Romano M. Helicobacter pylori and extragastric diseases: A review. World J Gastroenterol. 2018; 24(29):3204–3221. https://doi.org/10.3748/wjg.v24.i29.3204 PMID: 30090002

59. Ritter AMV, de Faria AP, Barbaro NR, Sabbatini AR, Corrêa NB, Brunelli V, et al. The rs243866/243865 polymorphisms in MMP-2 gene and the relationship with BP control in obese resistant hypertensive subjects. Gene. 2018; 646:129–135. https://doi.org/10.1016/j.gene.2017.12.023 PMID: 29288728

60. Misra S, Talwar P, Kumar A, Kumar P, Sagar R, Vibha D, et al. Association between matrix metallopro-teinase family gene polymorphisms and risk of ischemic stroke: A systematic review and meta-analysis of 29 studies. Gene. 2018; 672:180–194. https://doi.org/10.1016/j.gene.2018.06.027 PMID: 29906531

61. Yang W., Lu J., Yang L., Zhang J. Association of Matrix Metalloproteinase-9 gene -1562C/T polymor-phism with essential hypertension: a systematic review and meta-analysis article. Iran. J. Public Health. 2015; 44(11):1445–1452. PMID: 26744701

62. Moskalenko MI, Milanova SN, Ponomarenko IV, Polonikov AV, Churnosov MI. Study of associations of polymorphism of matrix metalloproteinases genes with the development of arterial hypertension in men. Kardiologiia. 2019; 59(7S):31–39. Russian. https://doi.org/10.18087/cardio.2598 PMID: 31441739

63. Polonikov A, Rymarova L, Klyosova E, Volkova A, Azarova I, Bushueva O, et al. Matrix metalloprotei-nases as target genes for gene regulatory networks driving molecular and cellular pathways related to a multistep pathogenesis of cerebrovascular disease. J Cell Biochem. 2019; 120(10):16467–16482. https://doi.org/10.1002/jcb.28815 PMID: 31056794

64. Jiang XC. Phospholipid transfer protein: its impact on lipoprotein homeostasis and atherosclerosis. J Lipid Res. 2018; 59(5):764–771. https://doi.org/10.1194/jlr.R082503 PMID: 29438986

65. Albers JJ, Vuletic S, Cheung MC. Role of plasma phospholipid transfer protein in lipid and lipoprotein metabolism. Biochim Biophys Acta. 2012; 1821(3):345–357. https://doi.org/10.1016/j.bbalip.2011.06.013 PMID: 21736953

66. Desrumaux C, Risold PY, Schroeder H, Deckert V, Masson D, Athias A, et al. Phospholipid transfer pro-tein (PLTP) deficiency reduces brain vitamin E content and increases anxiety in mice. FASEB J. 2005 Feb; 19(2):296–7. https://doi.org/10.1096/fj.04-2400fje PMID: 15576481

67. Zhou T, He Q, Tong Y, Zhan R, Xu F, Fan D, et al. Phospholipid transfer protein (PLTP) deficiency impaired blood-brain barrier integrity by increasing cerebrovascular oxidative stress. Biochem Biophys Res Commun. 2014; 445(2):352–6. https://doi.org/10.1016/j.bbrc.2014.01.194 PMID: 24513285

68. Bosmans LA, Bosch L, Kusters PJH, Lutgens E, Seijkens TTP. The CD40-CD40L Dyad as Immuno-therapeutic Target in Cardiovascular Disease. J Cardiovasc Transl Res. 2021; 14(1):13–22. https://doi.org/10.1007/s12265-020-09994-3 PMID: 32222950

69. Daub S, Lutgens E, Münzel T, Daiber A. CD40/CD40L and Related Signaling Pathways in Cardiovascu-lar Health and Disease-The Pros and Cons for Cardioprotection. Int J Mol Sci. 2020; 21(22):8533. https://doi.org/10.3390/ijms21228533 PMID: 33198327

70. Amini M, Ghorban K, Mokhtarzadeh A, Dadmanesh M, Baradaran B. CD40 DNA hypermethylation in primary gastric tumors; as a novel diagnostic biomarker. Life Sci. 2020; 254:117774. https://doi.org/10.1016/j.lfs.2020.117774 PMID: 32407843

71. Guo J, Xiao JJ, Zhang X, Fan KX. CD40 expression and its prognostic significance in human gastric carcinoma. Med Oncol. 2015; 32(3):63. https://doi.org/10.1007/s12032-014-0463-0 PMID: 25665853

72. Hunt MC, Alexson SE. The role Acyl-CoA thioesterases play in mediating intracellular lipid metabolism. Prog Lipid Res. 2002; 41(2):99–130. https://doi.org/10.1016/s0163-7827(01)00017-0 PMID: 11755680

73. Shaymardanova EKh Nurgalieva AKh, Khidiyatova IM Gabbasova LV, Kuramshina OA Kryukova AY, et al. Role of Allelic Genes of Matrix Metalloproteinases and Their Tissue Inhibitors in the Peptic Ulcer Disease Development. Genetika. 2016; 52(3):364–75. Russian. PMID: 27281857

74. Hu C, Weng F, Li L, Dai W, Yan J, Peng L, et al. Association between MMP-9–1562 C/T polymorphism and susceptibility to digestive cancers: A meta-analysis. Gene. 2018; 673:88–94. https://doi.org/10.1016/j.gene.2018.06.025 PMID: 29890308

75. Štrbac D, Goričar K, Dolžan V, Kovač V. Matrix Metalloproteinases Polymorphisms as Prognostic Bio-markers in Malignant Pleural Mesothelioma. Dis Markers. 2017; 2017:8069529. https://doi.org/10.1155/2017/8069529 PMID: 29138529

76. Oliveira VA, Chagas DC, Amorim JR, et al. Association between matrix metalloproteinase-9 gene polymorphism and breast cancer in Brazilian women. Clinics (Sao Paulo). 2020; 75:e1762. https://doi.org/10.6061/clinics/2020/e1762 PMID: 33146350

77. Kondkar AA. Updates on Genes and Genetic Mechanisms Implicated in Primary Angle-Closure Glaucoma. Appl Clin Genet. 2021; 14:89–112. Published 2021 Mar 9. https://doi.org/10.2147/TACG.S274884 PMID: 33727852

78. Zhao F, Fan Z, Huang X. Role of matrix metalloproteinase-9 gene polymorphisms in glaucoma: A hospital-based study in Chinese patients. Journal of clinical laboratory analysis, 2020; 34(3): e23105. https://doi.org/10.1002/jcla.23105 PMID: 31713905

79. Ling L, Li Y, Li H, Li W, Zhang HB. MMP-2 and MMP-9 gene polymorphisms act as biological indicators for ulinastatin efficacy in patients with severe acute pancreatitis. Medicine (Baltimore). 2019; 98(24): e15831. https://doi.org/10.1097/MD.0000000000015831 PMID: 31192912

80. Tziastoudi M, Stefanidis I, Hadjigeorgiou GM, Stravodimos K, Zintzaras E. A systematic review and meta-analysis of genetic association studies for the role of inflammation and the immune system in diabetic nephropathy. Clin Kidney J. 2017; 10(3):293–300. https://doi.org/10.1093/ckj/sfx008 PMID: 28616206

81. Li T, Zhang X, Sang L, Li XT, Sun HY, Yang J, et al. The interaction effects between TLR4 and MMP9 gene polymorphisms contribute to aortic aneurysm risk in a Chinese Han population. BMC Cardiovasc Disord. 2019; 19(1):72. https://doi.org/10.1186/s12872-019-1049-8 PMID: 30922233

82. Saravani S, Yari D, Saravani R, Azadi Ahmadabadi C. Association of COL4A3 (rs55703767), MMP-9 (rs17576) and TIMP-1 (rs6609533) gene polymorphisms with susceptibility to type 2 diabetes. Biomed Rep. 2017; 6(3):329–334. https://doi.org/10.3892/br.2017.856 PMID: 28451395