

Functionally significant polymorphisms of the *MMP-9* gene are associated with peptic ulcer disease in the Caucasian population of Central Russia

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Abstract

This study analyzed the association of functionally significant SNPs of matrix metalloproteinase (*MMP*) genes in the development of peptic ulcer disease (PUD) in Caucasians from Central Russia. Ten SNPs of the *MMP-1*, *MMP-2*, *MMP-3*, *MMP-8*, and *MMP-9* genes were analyzed for association with PUD in a cohort of 798 patients with PUD (including 404 *H. pylori*-positive and 394 *H. pylori*-negative) and 347 *H. pylori*-negative controls using logistic regression and assuming the additive, recessive, and dominant genetic models. Allele G of the rs17576 *MMP9* locus conferred a higher risk for PUD ($OR_{adj} = 1.31$, $p_{perm} = 0.016$), haplotype AACG of loci rs17576-rs3787268-rs2250889-rs17577 of the *MMP9* gene decreased risk for PUD ($OR_{adj} = 0.17$, $p_{perm} = 0.003$). Also, allele C of rs3918249, allele G of rs17576 and haplotype CG of rs3918249-rs17576 of the *MMP9* gene increased risk for *H. pylori*-positive PUD ($OR_{adj} = 1.82$, $p_{perm} = 0.002$; $OR_{adj} = 1.53$ – 1.95 $p_{perm} = 0.001$ – 0.013 and $OR_{adj} = 1.49$ $p_{perm} = 0.009$ respectively). The above loci and 50 linked to them possess significant regulatory effects and may affect the alternative splicing of four genes and the expression of 17 genes in various organs and tissues related to the PUD pathogenesis.

Introduction

Peptic ulcer is the cyclical appearance of a limited mucosal defect in the digestive tract (usually the stomach or duodenum) extending deeply beyond the muscular plate of the mucous membrane, with inflammatory infiltration and thrombotic necrosis in adjacent tissues¹. The prevalence of peptic ulcer disease (PUD) in the general population is estimated at 5–10%². Symptoms of PUD are variable and may include epigastric pain on an empty stomach and after eating, heartburn, nausea, vomiting, weight loss, and bleeding or perforation with the complicated disease^{1–3}. Identifying the risk factors and mechanisms of PUD helps to develop efficient strategies for diagnostics and treatment¹.

Mucosal defects in patients with the acid peptic disease have been traditionally considered as a result of increased gastric acid secretion in the stomach and degradation of the mucus barrier^{2,4}. Risk factors for PUD, including gastric and duodenal ulcers, are infection by *H. pylori*, alcohol and tobacco consumption, use of non-steroidal anti-inflammatory drugs (NSAIDs) and aspirin, stress, etc.^{2–6}. However, only a relatively small proportion of people infected by *H. pylori* or using NSAIDs develop PUD that suggests variation in individual susceptibility to the beginning of mucosal damage⁷. On the other hand, about one-fifth of cases include *H. pylori*-negative, NSAID-negative, and aspirin-negative PUD collectively classified as an idiopathic ulcer⁸. This type of ulcer is thought to occur due to the imbalance between factors important for mucosal integrity and aggressive insults, but the exact pathogenic mechanisms of idiopathic peptic ulcer remain unknown³.

Matrix metalloproteinases (MMPs) are endopeptidases playing an important role in the extracellular matrix (ECM) remodeling, cell proliferation, and inflammation. MMPs are synthesized and secreted by gastric and duodenal epithelial cells, macrophages, and neutrophils¹³. Since ECM degradation is an important factor of gastric and duodenal mucosal damage and subsequent PUD, MMPs play a key role in this process^{12,13,14}. There is evidence that cleaving and remodeling of the ECM by MMPs is one of the factors contributing to gastric ulceration (GU)^{15,16}. The role of several MMPs (MMP-1, MMP-2, MMP-3, MMP-9, and MMP-13) in GU was studied using animal models^{16–19}. MMP-9 was shown to be important in the early phase of chronic GU¹⁹.

Several genes have been reported for their association with peptic ulcers^{7,9–11}. Polymorphisms of the *MMPs* genes (*MMP-9*, *MMP-7*, *MMP-3*) may contribute to a genetic risk profile for gastric and duodenal ulcers in chronic *H. pylori* infection^{9–11}. *H. pylori* infection can induce the expression of MMP-3, MMP-7, and MMP-9 in the gastric mucosa and sera^{10,20,21}. *MMP-9* was significantly up-regulated in *H. pylori*-positive as compared to *H. pylori*-negative GU²².

Despite the apparently significant role of MMPs in PUD pathogenesis, associations of MMP genetic variants with PUD have been poorly analyzed: only a few studies of this problem have been published so far^{9–11,23}. This prompts for filling in this gap.

The present study analyzed polymorphisms of *MMP-1*, *MMP-2*, *MMP-3*, *MMP-8*, and *MMP-9* genes for their association with PUD and possible role in the susceptibility to the disease in the Caucasian sample from the Central Region of Russia.

Results

The phenotypic data of the study participants are shown in Table 1. The PUD patients had a more common family history of peptic ulcer ($p = 0.0005$), alcohol ($p = 0.0005$) and tobacco ($p = 0.0005$) consumption, stress ($p = 0.0005$), the presence of cardiovascular pathology ($p = 0.0005$) versus control group. These parameters were used as confounding factors (covariates) in the regression association analyses.

Table 1
Phenotypic characteristics of the study participants.

Parameters	Control	PUD	p
	mean \pm SD, % (n)	mean \pm SD, % (n)	
N	347	798	-
Age, years (min-max)	48.47 \pm 13.69 (22-79)	48.54 \pm 14.28 (20-79)	0.92
Gender ratio, f/m	66.28/33.72 (230/117)	67.42/32.58 (538/260)	0.76
BMI, kg/m ²	26.83 \pm 5.09	26.94 \pm 5.30	0.78
Age of developing peptic ulcer, years	-	41.12 \pm 12.87	-
Family history of peptic ulcer	4.32 (15)	18.29 (146)	0.0005
Current smoking	14.99 (52)	33.08 (264)	0.0005
Alcohol consumption	32.28 (112)	51.13 (408)	0.0005
Stress	37.17 (129)	77.19 (616)	0.0005
Positivity <i>H. pylori</i> test (endoscopic biopsy and histological identification)	-	50.63 (404)	-
PUD characteristics			
Location	-	2.76 (22)	-
Stomach: Body	-	3.01 (24)	-
Pylorus	-	48.62 (388)	-
Antrum	-	45.61 (364)	-
Duodenum: Bulb	-	-	-
Sizes ulcer (diameter) (cm)	-	0.61 \pm 0.40	-
Sizes ulcer: Small (< 0.5 cm)	-	45.37 (362)	-
Medium (0.5-1.0 cm)	-	44.86 (358)	-
Large (> 1.0 cm)	-	9.77 (78)	-
PUD associated complications			
Bleeding	-	3.51 (28)	-
Perforation	-	8.27 (66)	-
Stenosis	-	6.52 (52)	-
Malignancy	-	2.26 (18)	-
Other somatic pathologies			
Cardiovascular pathology	26.80 (93)	48.37 (386)	0.0005
Endocrine pathology	3.17 (11)	5.01 (40)	0.22
Kidney pathology	2.59 (9)	3.76 (30)	0.41
Respiratory system pathology	4.32 (15)	5.76 (46)	0.39
Nervous system pathology	7.78 (27)	9.52 (76)	0.40
Musculoskeletal system pathology	6.91 (24)	8.02 (64)	0.60
p values < 0.05 are shown in bold.			

Supplementary Table S1 shows distributions of genotypes and alleles of the ten studied SNPs in the PUD patients and control groups. All analyzed SNPs were in the HWE ($p > 0.005$, $p_{\text{bonf}} > 0.05$). After the Bonferroni correction, only polymorphisms of the *MMP9* gene manifested association with PUD (Table 2). Specifically, the increased risk of PUD was associated with allele G of SNP rs17576 (additive model, the odds ratio adjusted for confounding factors $OR_{\text{adj}} = 1.31$, $p_{\text{perm}} = 0.016$, power – 82.98%) (Table 2). Two loci were associated with *H. pylori*-positive PUD (rs3918249 and rs17576) (Table 3). Allele C of SNP rs3918249 showed a significant association with the increased risk of *H. pylori*-positive PUD (dominant model, $OR_{\text{adj}} = 1.82$, $p_{\text{perm}} = 0.002$, power – 96.43%). The increased risk of *H. pylori*-positive PUD was also associated with a carriage of allele G of loci rs17576 according to the all three genetic models: additive ($OR_{\text{adj}} = 1.53$, $p_{\text{perm}} = 0.001$, power – 98.14%), dominant ($OR_{\text{adj}} = 1.67$, $p_{\text{perm}} = 0.013$, power – 90.21%), recessive ($OR_{\text{adj}} = 1.95$, $p_{\text{perm}} = 0.007$, power – 94.75%).

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

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		L95	U95
rs1940475	<i>MMP-8</i>	T	1136	0.96	0.79	1.18	0.708	0.91	0.66	1.26	0.573	0.99	0.71	1.39	0.960
rs1799750	<i>MMP-1</i>	2G	1107	0.89	0.73	1.09	0.263	0.86	0.62	1.19	0.362	0.84	0.59	1.02	0.345
rs679620	<i>MMP-3</i>	T	1133	0.97	0.79	1.20	0.797	0.93	0.66	1.30	0.655	1.01	0.72	1.41	0.979
rs243865	<i>MMP-2</i>	T	1121	0.96	0.76	1.22	0.749	0.94	0.69	1.27	0.672	1.01	0.57	1.80	0.969
rs3918242	<i>MMP-9</i>	T	1127	1.00	0.75	1.32	0.973	1.06	0.77	1.46	0.733	0.58	0.22	1.52	0.266
rs3918249	<i>MMP-9</i>	C	1125	1.16	0.93	1.43	0.181	1.45	1.07	1.97	0.018	0.88	0.59	1.33	0.549
rs17576	<i>MMP-9</i>	G	1140	1.31	1.05	1.60	0.016	1.35	0.99	1.83	0.054	1.51	1.00	2.27	0.048
rs3787268	<i>MMP-9</i>	A	1133	1.12	0.87	1.45	0.384	1.17	0.86	1.58	0.315	1.02	0.48	2.14	0.968
rs2250889	<i>MMP-9</i>	G	1128	0.79	0.57	1.09	0.148	0.77	0.53	1.12	0.172	0.63	0.22	1.80	0.388
rs17577	<i>MMP-9</i>	A	1112	1.00	0.75	1.32	0.988	1.01	0.80	1.52	0.563	0.46	0.18	1.17	0.102
All results were obtained after adjustment for covariates.															
OR, odds ratio.															
95% CI, 95% confidence interval.															
p values < 0.017 are shown in bold.															

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

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			
<i>H. pylori</i> -positive PUD															
rs1940475	<i>MMP-8</i>	T	744	0.97	0.76	1.23	0.774	0.91	0.62	1.36	0.656	0.99	0.66	1.49	0.979
rs1799750	<i>MMP-1</i>	2G	725	0.88	0.69	1.13	0.313	0.83	0.56	1.23	0.361	0.85	0.55	1.31	0.452
rs679620	<i>MMP-3</i>	T	743	0.92	0.71	1.18	0.505	0.85	0.57	1.28	0.447	0.93	0.62	1.42	0.744
rs243865	<i>MMP-2</i>	T	735	0.98	0.74	1.30	0.879	0.90	0.63	1.30	0.588	1.26	0.64	2.46	0.509
rs3918242	<i>MMP-9</i>	T	739	1.17	0.83	1.63	0.376	1.34	0.92	1.96	0.127	0.30	0.06	1.39	0.123
rs3918249	<i>MMP-9</i>	C	737	1.33	1.03	1.72	0.031	1.82	1.23	2.67	0.002	1.03	0.63	1.67	0.914
rs17576	<i>MMP-9</i>	G	746	1.53	1.19	1.98	0.001	1.67	1.14	2.43	0.008	1.95	1.22	3.11	0.005
rs3787268	<i>MMP-9</i>	A	745	1.23	0.91	1.67	0.181	1.26	0.87	1.81	0.219	1.43	0.62	3.30	0.396
rs2250889	<i>MMP-9</i>	G	736	0.77	0.51	1.15	0.203	0.78	0.49	1.23	0.282	0.42	0.09	2.01	0.280
rs17577	<i>MMP-9</i>	A	728	1.20	0.86	1.68	0.271	1.43	0.98	2.09	0.067	0.37	0.10	1.35	0.132
<i>H. pylori</i> -negative PUD															
rs1940475	<i>MMP-8</i>	T	738	0.98	0.77	1.25	0.893	0.94	0.63	1.39	0.752	1.02	0.68	1.53	0.920
rs1799750	<i>MMP-1</i>	2G	721	0.91	0.71	1.17	0.459	0.90	0.61	1.33	0.596	0.86	0.56	1.32	0.483
rs679620	<i>MMP-3</i>	T	735	0.86	0.67	1.10	0.235	0.79	0.53	1.19	0.258	0.84	0.55	1.28	0.419
rs243865	<i>MMP-2</i>	T	729	0.94	0.70	1.26	0.696	0.97	0.67	1.39	0.849	0.80	0.39	1.67	0.558
rs3918242	<i>MMP-9</i>	T	731	0.83	0.59	1.18	0.296	0.80	0.53	1.20	0.276	0.83	0.28	2.46	0.739
rs3918249	<i>MMP-9</i>	C	733	1.00	0.78	1.30	0.975	1.15	0.80	1.66	0.448	0.77	0.46	1.30	0.323
rs17576	<i>MMP-9</i>	G	740	1.08	0.83	1.40	0.569	1.09	0.76	1.57	0.626	1.12	0.67	1.88	0.660
rs3787268	<i>MMP-9</i>	A	733	1.01	0.74	1.40	0.930	1.09	0.75	1.57	0.656	0.62	0.21	1.77	0.367
rs2250889	<i>MMP-9</i>	G	734	0.81	0.55	1.20	0.293	0.78	0.49	1.22	0.268	0.82	0.25	2.07	0.739
rs17577	<i>MMP-9</i>	A	724	0.80	0.57	1.14	0.217	0.81	0.54	1.22	0.310	0.53	0.17	1.65	0.270
All results were obtained after adjustment for covariates.															
OR, odds ratio.															
95% CI, 95% confidence interval.															
p values < 0.017 are shown in bold.															

Haplotype AACG defined by rs17576-rs3787268-rs2250889-rs17577 was associated with PUD ($OR_{adj}=0.17$, $p = 0.001$, $p_{perm}=0.003$), haplotype CG defined by rs3918249-rs17576 of the *MMP9* gene was associated with *H. pylori*-positive PUD ($OR_{adj}=1.49$, $p = 0.004$, $p_{perm}=0.009$) (Fig. 1). Thus, in total five polymorphisms of the *MMP9* gene were associated with PUD (two individually and three within haplotypes).

Functional SNP

Non-synonymous SNPs. Among the PUD-associated SNPs, three polymorphisms (rs17576, rs2250889, and rs17577) were missense (Supplementary Table S2).

Regulatory effects. The data on the regulatory effects of the PUD-associated loci of the *MMP9* gene are presented in Supplementary Table S3. According to the HaploReg database, three SNPs were located in evolutionarily conserved regions, all five polymorphisms - in the region of DNA binding with modified histone (H3K4me3, H3K9ac) marking promoters and hypersensitivity region to DNase-1 in various tissues, four SNPs - in

the region of DNA binding with modified histone (H3K4me1, H3K27ac) marking enhancers and two polymorphisms - in the protein-bound region. Importantly, the PUD-associated SNPs manifest their regulatory effects in the tissues and organs related to the pathogenesis of the disease (fetal stomach and small intestine, adult gastric and small intestine, adult stomach and duodenum mucosa, etc.).

In addition to the five PUD-associated SNPs, regulatory significance was estimated for 50 polymorphisms linked to them (Supplementary Table S3). Three synonymous SNPs were located in exons of the *MMP9* gene, 28 SNPs were in 5'-UTR of the *MMP9*, *ZNF335*, and *SLC12A5* genes, 19 were in introns. Ten loci were located in evolutionarily conserved regions. The *in silico* analysis of the linked SNPs suggested several polymorphisms with pronounced regulatory effects (Supplementary Table S3). For example, rs3848722 (was in linkage disequilibrium with SNPs rs3918249 and rs17576) is located in the hypersensitive region to DNAase-I (19 tissues), in the region of DNA binding with modified histone marking promoters and enhancers (5 and 14 tissues respectively), and a putative transcription factor binding sites (Pax-6, HNF4, ZID, NRSF).

Expression QTLs. *In silico* analysis for the eQTL impact of the PUD-associated SNPs shows they might affect the expression of 17 genes (*MMP9*, *CD40*, *NTTIP1*, *NEURL2*, *PCIF1*, *PLTP*, *RP11-465L10.10*, *RP3-337O18.9*, *RPL13P2*, *SLC12A5*, *SNX21*, *SPATA25*, *SYS1*, *WFDC10B*, *WFDC3*, *ZNF335*, *ZSWIM1*) in more than 20 tissues and organs (Supplementary Table S4). The PUD-associated loci were also in strong LD with the 48 SNPs affecting the expression of the above 17 genes in various organs and tissues (Supplementary Table S5).

Splicing QTLs. The PUD-associated SNPs had possess the potential impact on the genes alternative splicing and might sQTL affect for 4 genes (*PLTP*, *ACOT8*, *SNX21*, *SLC12A5*) (Supplementary Table S6). These loci were tightly linked to 48 polymorphisms affecting sQTL of the above four genes in more than 20 tissues and organs (Supplementary Table S7).

Discussion

The present study reports for the first time the association of *MMP-9* gene polymorphisms with PUD in Caucasians from Central Russia: allele G of SNPs rs17576 locus increased risk for PUD ($OR_{adj} = 1.31$) whereas haplotype AACG of rs17576-rs3787268-rs2250889-rs17577 decreased the risk ($OR_{adj}=0.17$). Also, allele C of rs3918249, allele G of the rs17576 and haplotype CG of rs3918249-rs17576 increased risk for the *H. pylori*-positive PUD ($OR_{adj} = 1.82$, $OR_{adj} = 1.53-1.95$ and $OR_{adj} = 1.49$ respectively). The PUD-associated loci appeared to possess a significant regulatory effects and influence the expression of 17 genes and alternative splicing of four genes.

One of the PUD-associated loci, rs17576, was previously shown as a candidate for *H. pylori*-positive gastric ulcer¹¹, peptic ulcer, and *H. pylori*-positive peptic ulcer⁹. However, the data about the risk alleles of this locus were contradictory, Specifically, Shaimardanova et al.⁹ reported allele G (i.e., the same as determined in the present study) as the risk factor for PUD and *H. pylori*-positive PUD in Tatars from the Bashkortostan Republic of Russia, whereas Hellmig et al.¹¹ determined allele A as the risk factor for *H. pylori*-positive gastric ulcer in Germans. On the other hand, Yeh Y.C. et al.¹⁰ did not find any association of rs17576 with either gastric or duodenal ulcer after *H. pylori* infection in Taiwanese. Okada R. et al.²⁴ ported the association of rs17576 *MMP9* c gastric cancer both individually and within haplotype CAA rs3918242-rs17576-rs17577 of the *MMP-9* gene.

The MMP-9 protein (gelatinase B) cleaves denatured collagen and plays a significant role in ECM modification²⁵. MMPs can be induced by both *H. pylori* bacterial products and proinflammatory cytokines²⁶. Overexpression of MMPs may result in extracellular matrix breakdown and tissue disintegration. Li et al.²² reported higher MMP-9 expression in the gastric mucosa at the boundary of the gastric ulcer. Significantly elevated expression of pro-MMP9 (about 12-fold) was documented in the indomethacin-induced gastric ulcer as compared to unaffected tissues. Ethanol produced an even stronger effect and increased pro-MMP-9 expression in rat gastric tissues up to 22-fold¹⁷. Overexpression of MMP-9 in indomethacin-induced gastric ulcer in mice correlated with up-regulation of activator protein-1 and preceded oxidative stress²⁷.

During PUD, the gastric and duodenal mucosa is infiltrated by monocytes, lymphocytes, neutrophils, and plasma cells. Inflammatory cells produce multiple pro-inflammatory cytokines and growth factors (e.g., epidermal growth factor, transforming growth factor- β , platelet-derived growth factor, vascular endothelial growth factor, etc.)^{22,22}. Pro-inflammatory cytokines can elevate the expression of MMPs²⁶. Chronic inflammation precedes oxidative stress and increases the expression of MMP-9²².

We determined associations of the *MMP-9* gene polymorphisms with *H. pylori*-positive PUD but did not find the association of any of the analyzed *MMP* genes with *H. pylori*-negative PUD. The polymorphisms of the *MMP-9* gene may contribute to a complex genetic risk profile of PUD in chronic *H. pylori* infection^{9,11}. Our results are in agreement with the previous reports about more significant contribution of *MMP-9* to the development of *H. pylori*-positive gastric ulcer and gastritis as compared to the other *MMP* genes^{22,28-30}. Li et al.²² showed that *MMP-9* expression levels in the gastric mucosa were significantly elevated in *H. pylori*-positive gastric ulcer patients as compared to the *H. pylori*-negative ones and correlated with the histologically determined activity level and inflammation at the boundary of the ulcer. Epithelium of the *H. pylori*-induced gastric ulcer manifested higher *MMP-9* expression than that of the NSAID-related gastric ulcer²⁸. Significantly higher serum levels

of MMP-9 were determined in patients with *H. pylori*-positive gastritis as compared to *H. pylori*-negative controls²⁹. Antral mucosa of *H. pylori*-infected patients with gastritis demonstrated a 19-fold higher MMP-9 protein activity and 10-fold increase of *MMP-9* gene expression of than that of uninfected individuals³⁰. Successful treatment of the *H. pylori* infection lowered the *MMP-9* expression levels, whereas the elevated levels remain unchanged when the treatment failed³¹.

It should be noted that the current study is somewhat limited because only one ethnic population was analyzed. The well-known ethnic disparities in the prevalence of complex diseases warrant validation studies of the determined associations of the *MMP* genes and PUD in other ethnic populations.

Conclusions

Genetic variants of the gene are associated with PUD in a population of Central Russia. However, the data about possible role of the *MMP* genes polymorphic variants in the susceptibility to PUD in different ethnic populations remain inconsistent that warrants further studies to identify possible causative variants for the disease.

Methods

Study subjects

In total, 1145 participants of Russian origin and born in Central Russia^{32,22}, including 798 patients with PUD (434 with gastric ulcer and 364 with duodenal ulcer), and 347 controls, were recruited for the study. PUD and complications (if any) were determined on the basis of conventional clinical and endoscopic findings. The control group consisted of healthy individuals with no symptoms of gastrointestinal disease³⁴. They were not examined by endoscopy because, apart from ethical reasons, the chance of finding an active ulcer in patients without symptoms was very low³⁵. Patients and control group volunteers that used NSAIDs, corticosteroids, and aspirin for a long-term treatment were excluded.

The *H. pylori* infection in patients was diagnosed by detection of the pathogen in biopsies obtained during endoscopy. Among 798 patients with PUD, 404 were *H. pylori*-positive and 394 were *H. pylori*-negative. In the controls, the presence of *H. pylori* was diagnosed by the serological test using a commercial IgG ELISA kit (Plate Helicobacter IgG, Roche). Control group volunteers diagnosed with *H. pylori* infection were excluded from the study.

The study protocol was approved by the Medical Institution Ethics Committee of Belgorod State University. All participants signed an informed consent prior to enrolment in the study. All methods were performed in accordance with the relevant guidelines and regulations. The participants took the medical examination at the Department of Gastroenterology of St. Joasaph Belgorod Regional Clinical Hospital.

Isolation of DNA and genotyping

A blood sample (4–5 ml) was collected by venipuncture from all study participants in EDTA-coated tubes (Vacutainer®). Genomic DNA was isolated from the buffy coat using a standard phenol/chloroform procedure (as described earlier³⁶).

Ten SNPs of the *MMP* genes (rs1799750 *MMP-1*, rs243865 *MMP-2*, rs679620 *MMP-3*, rs1940475 *MMP-8*, rs3918242, rs3918249, rs3787268, rs2250889, rs17576, and rs17577 *MMP-9*) were selected for the analysis according to the following criteria^{37,38}: previously reported associations with digestive diseases (PUD, gastric cancer, etc.), regulatory potential, and MAF > 0.05.

All selected SNPs had significant regulatory potential as evidenced by the HaploReg online tools³⁹ (Supplementary Table S8); eight polymorphisms were associated with digestive diseases (PUD, gastric and esophageal cancer, digestive cancers, gastritis) (including two SNPs associated with PUD) in previously published candidate gene association studies (Supplementary Table S9). Two SNPs (rs3918249 and rs3787268 *MMP-9*) did not demonstrate a significant association with digestive diseases but had significant regulatory potential (according to HaploReg).

The polymorphisms were genotyped using the MALDI-TOF mass spectrometry iPLEX platform (Agena Bioscience Inc, San Diego, CA). The quality was controlled by genotyping of blind replicates⁴⁰. Regenotyping of 5% of the studied samples, selected on a random basis, showed 100% reproducibility of the original results.

Statistical analysis

The observed allele and genotype frequencies were assessed for correspondence to the Hardy-Weinberg equilibrium using the chi-square test. Associations of the SNPs with PUD were analyzed by logistic regression according to three main genetic models, additive, recessive, and dominant⁴¹. The regression analysis was adjusted for covariates: family history of peptic ulcer, alcohol and tobacco consumption, stress, the presence of cardiovascular pathology were used as qualitative variables (Table 1). The haplotype blocks were constructed for *MMP-9* gene

variants using the «Solid Spine» algorithm ($D' > 0.8$) by HaploView program⁴². The logistic regression analyses and adaptive permutation test to adjust for multiple comparisons⁴³ were calculated by using the PLINK software⁴⁴. P_{perm} -value ≤ 0.017 was set to be statistically significant (after the Bonferroni correction based on the numbers of paired comparisons, $n = 3$: PUD – control, *H. pylori*-positive PUD - control, and *H. pylori*-negative PUD - control).

Functional SNPs

The polymorphisms associated with PUD and those strongly linked to them ($r^2 \geq 0.8$) were analyzed for their functional significance (non-synonymous SNPs, regulatory potential, eQTLs, and sQTLs)⁴⁵. SNPs in strong linkage disequilibrium (LD) with the PUD-associated variants were identified using HaploReg³⁹. Non-synonymous SNPs and their functional predictions were analyzed using the SIFT online tool⁴⁶. The regulatory impact of the candidate *MMP* loci for PUD was evaluated by using HaploReg³⁹. The effects of the investigated SNPs on the mRNA levels and splicing QTLs was estimated using the GTEx project data⁴⁷ and the FDR ≤ 0.05 as the significance level. Likewise, eQTL and sQTL values of polymorphisms in strong LD ($r^2 \geq 0.8$) with the PUD-associated loci were estimated⁴⁸.

Declarations

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions

OM, VD, MC substantial contributions to conception and design. OM, ER acquisition of data. IP, VD analysis and interpretation of data. OM, IP, drafting the article. ER, VD, MC revising it critically for important intellectual content. All authors final approval of the version to be published.

All authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

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Figures

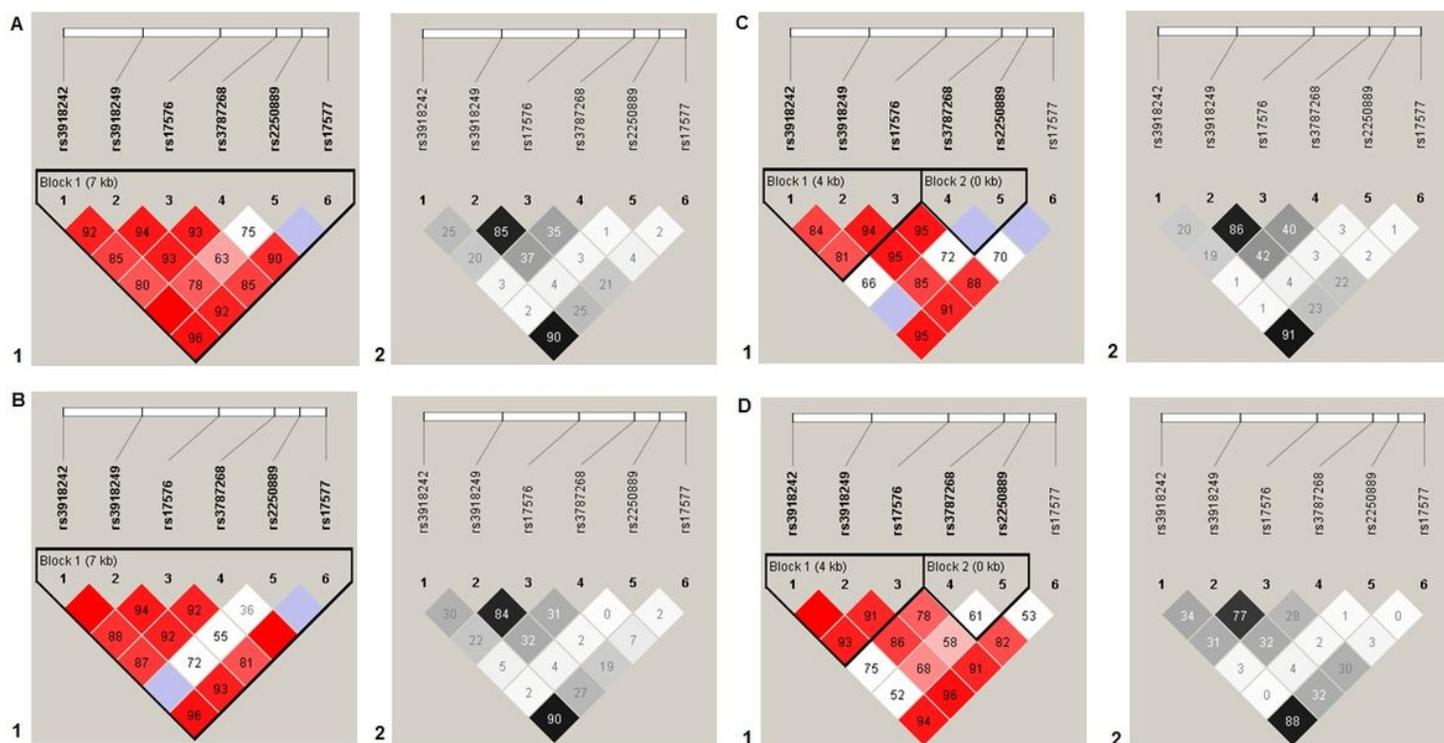


Figure 1

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

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