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HUMAN GENETICS

Association of Cytokine Gene Polymorphisms in Peptic Ulcer Development in the Bashkortostan Republic

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Abstract—Peptic ulcer disease (PUD) is a chronic disease based on recurrent gastric or duodenal ulcers. Association analysis of common polymorphisms of the cytokines genes *IL1B* (*rs1143634*), *IL1RN* (*rs71941886*), *IL8* (*rs4073*), *IL10* (*rs1800872*), and *TNFA* (*rs1800629*) was conducted in 254 patients with gastric ulcer or duodenal ulcer and in 277 unrelated healthy individuals from the Bashkortostan Republic. Associations of the *rs1143634*C* allele and the *C/C* genotype of the *IL1B* gene with PUD in ethnic Bashkirs have been revealed. The frequency of the *rs4073*A/A* genotype of the *IL8* gene was significantly higher in the control group as compared to patients infected with *H. pylori*. Our results confirm the significant role of *rs1143634* of the *IL1B* gene in PUD development.

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INTRODUCTION

Peptic ulcer disease (PUD) is a chronic recurrent disease characterized by the alternation of exacerbation and remission periods and the formation of a defect (ulcer) in the stomach and (or) duodenum wall that penetrates into the submucosal layer [1]. PUD represents one of the most frequent diseases of the gastrointestinal tract, with a frequency about 10% worldwide. PUD might result in the loss of work capacity or even in lethality: mortality from this pathology varies in different countries, from 6 to 7.1 per 100000 for gastric ulcer and from 0.2 to 9.7 for duodenal ulcer. According to various data, 1.5–3 million adults, 18 thousand adolescents, and about 10 thousand children under the age of 14 in Russia possess PUD. The frequency of duodenal ulcer is four times higher than the frequency of gastric ulcer [1, 2].

The most frequent reason for PUD development is infection by *Helicobacter pylori*, which is detected in the majority of patients [1, 3, 4]. This bacterium infected more than 50% of the population in various countries; however, only 10–15% of them develop a gastric or duodenal ulcer [1, 4]. Assuming a high level of infection by *H. pylori* in distinct populations, a significantly higher level of morbidity should be expected. *H. pylori* ulcerogenecity undoubtedly depends on a large number of endogenous and exogenous risk factors. Neuro-emotional overstressing, unfavorable nutrition, smoking, alcohol, and several pharmaceuticals represent unfavorable factors increasing the risk for pathology development. Moreover, an ulcer might develop on the basis of chronic gastritis, functional gastric and duodenal abnormalities, or increased acidity of gastric juice, as well as from heritability [1–4].

H. pylori infection is known to be accompanied by an increased secretion of several cytokines, such as tumor necrosis factor alpha and interleukin 1, 2, 6, 8, and 10, that determine the activation and suppression of the immune response toward infectious agents, including helicobacter, on the gastric and duodenal mucous membrane. Polymorphic variants of genes encoding for immune-mediator proteins might determine the variability of their structure and expression and, hence, immune response reactivity toward infectious agents [5–8]. Therefore, the study of polymorphisms of the key pro- and anti-inflammatory cytokine genes for the assessment of their role in PUD development remains urgent.

The present study aimed to study the association of polymorphic variants of interleukin-1β *IL1B* (*rs1143634*), interleukin 1 receptor antagonist *IL1RN* (*rs71941886*), interleukin 8 *IL8* (*rs4073*), interleukin 10 *IL10* (*rs1800872*), and tumor necrosis factor α *TNFA* (*rs1800629*) genes with PUD in Bashkortostan Republic (RB).

MATERIALS AND METHODS

The material for the study included DNA samples obtained from individuals with PUD and healthy donors aged 18–80 years from Ufa, RB. The patient group was composed of 254 individuals (212 diagnosed with duodenal ulcer and 42 with gastric ulcer

Table 1. Polymorphic variants, primer sequences, and allele nomenclature of the studied DNA loci

Gene	Polymorphism dbSNP	Primers, 5'–3'	Restriction endonuclease, alleles, fragment sizes, bp
IL1B 2q13	*3953C>T* *rs1143634*	GTTGTCATCAGACTTTGACC TTCAGTTCATATGGACCAGA	*Taq*I *C*, 190 + 114 *T*, 304
IL4RN 2q14.2	*VNTR* *rs71941886*	TCCTGGTCTGCAGGTAA CTCAGCAACACTCCTAT	*1*, 412 *2*, 240 *3,* 498 *4*, 326
IL8 4q13-q21	*–251T>A* *rs4073*	TTGGCTGGCTTATCTTCACC GAGGAAATTCCACGATTTGC	*Mfe*I *T*, 350 *A,* 183 + 167
IL10 1q31-q32	*–627C>A* *rs1800872*	CCTAGGTCACAGTGACGTGG GGTGAGCACTACCTGACTAGC	*Rsa*I *A*, 236 + 176 *C*, 412
TNFA 6p21.3	*–308G>A* *rs1800629*	AGGCAATAGGTTTTGAGGGCCAT TCCTCCCTGCTCCGATTCGG	*Nco*I *G*, 87 + 20 *A*, 107

and mixed PUD forms) of various ethnicity (62 Russians, 104 Tatars, 61 Bashkirs, and 27 individuals of mixed origin) from the clinical departments of polyclinic numbers 1, 5, 46, 47, 49, 50 of Ufa. Of those, 120 patients were characterized by infection with *H. pylori*, which was confirmed via a urease test, as well as via serological and histological analyses. Healthy donors without any diseases of gastrointestinal tract, 277 individuals of different ethnic background (134 Russians, 100 Tatars, 31 Bashkirs, and 12 individuals of mixed origin), represent the control group. The gender distribution was the following: 207 men and 47 women in the patient groups and 203 men and 74 women in the control group.

Genomic DNA was isolated from peripheral blood lymphocytes via the phenol-chloroform extraction technique [9]. Amplification of the studied polymorphisms was carried out via polymerase chain reaction of DNA synthesis on a GeneAmp PCR System 2720 (Applied Biosystems, United States). Nucleotide substitutions were detected via restriction fragment length polymorphism (RFLP) analysis. The list of investigated loci, primers sequences, sizes of amplified fragments, and restriction endonucleases are shown in Table 1 [10–13]. The results of RFLP-analysis were detected via electrophoresis in 7% polyacrylamide gel, followed by staining with ethidium bromide and visualization in passing UV light. Statistical analysis was performed using MS Office Excel. For pairwise comparison of allele and genotype frequencies among patients and control groups, χ^2-criteria for contingency tables 2×2 with Yates's correction for continuity was used (http://www.biometrica.tomsk.ru/). In the case of statistically significant differences ($p < 0.05$) between the studied groups, the odds ratio (OR) and 95% confidence intervals (CI 95%) were calculated [14].

Meta-analysis was performed on the basis of results revealed from Russian, Tatar, and Bashkir populations via WinPepi v. 11.32 software (http://www.brixton-health.com/pepi4windows.html) [15]. Models with fixed (Mantel–Haenszel method) and random effects (Dersimonian–Laird method) were used for the calculation of the mean OR and significance level. For the estimation of statistical heterogeneity of various samples, the I^2 criterion (part of variability explained by samples heterogeneity) was used [16]. Heterogeneity was assumed as low for $I^2 < 30\%$, moderate for I^2 within 30–50%, and high for $I^2 > 50\%$.

Gene-gene interaction was conducted via GMDR (Generalized Multifactor-Dimensionality Reduction) software [17].

RESULTS

An analysis of the allele and genotype frequencies of several polymorphic variants of interleukin-1β *IL1B* (*rs1143634*), interleukin 1 receptor antagonist *IL1RN* (*rs71941886)*, interleukin 8 *IL8* (*rs4073*), interleukin 10 *IL10* (*rs1800872*), and tumor necrosis factor α *TNFA* (*rs1800629*) genes was conducted in PUD patients and control individuals from the Bashkortostan Republic. The observed distribution of genotype frequencies was in accordance with that expected in accordance with the Hardy–Weinberg equilibrium. In light of the ethnical heterogeneity in the population from RB, individuals from the most frequent ethnic groups, such as Russians, Tatars, and Bashkirs, were included in the analysis. In order to detect markers of increased and decreased risk of PUD development, a comparison between PUD

Table 2. Distribution of allele and genotype frequencies of *rs1143634* polymorphism of the *IL1B* gene in patients with PUD and healthy donors

Sample		Genotypes			Alleles		*N*
		CC	*CT*	*TT*	*C*	*T*	
		n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	
Patients with PUD	Russians	35 (58.33)	19 (31.67)	6 (10.00)	89 (74.17)	31 (25.83)	60
	Tatars	62 (64.58)	29 (30.21)	5 (5.21)	153 (79.69)	39 (20.31)	96
	Bashkirs	42 (71.19)	15 (25.42)	2 (3.39)	99 (83.90)	19 (16.10)	59
	Duodenal ulcer	123 (61.81)	63 (31.66)	13 (6.53)	309 (77.64)	89 (22.36)	199
	H. pylori	67 (59.82)	37 (33.04)	8 (7.14)	171 (76.34)	53 (23.66)	112
	In total	150 (62.24)	76 (31.54)	15 (6.22)	376 (78.00)	106 (22.00)	241
Control	Russians	61 (46.57)	55 (41.98)	15 (11.45)	177 (67.56)	85 (32.44)	131
	Tatars	58 (59.79)	32 (32.99)	7 (7.22)	148 (76.29)	46 (23.71)	97
	Bashkirs	11 (35.48)	18 (58.06)	2 (6.45)	40 (64.52)	22 (35.48)	31
	In total	135 (49.63)	113 (41.54)	24 (8.83)	383 (70.40)	161 (29.60)	272

n quantity; *N* sample size. The same for Tables 2–6.

patients and control individuals of corresponding ethnicity was conducted for the allele and genotype frequency distributions of polymorphic DNA loci.

The allele and genotype frequencies of *IL1B rs1143634* (*3953C>T*) are demonstrated in Table 2. Significant differences in the allele and genotype frequency distributions were observed between control individuals of Russian, Tatar and Bashkir ethnicity. The highest frequency of the common *rs1143634*C* allele was detected in 76.29% cases in healthy ethnic Tatars as compared to healthy Russians (in 67.56% of cases) ($\chi^2 = 4.15$, $p = 0.04$). The frequency of the *rs1143634*C* allele in healthy Bashkirs was 64.52%. The distribution of genotype frequencies also appeared to be nonuniform between the described groups: the frequency of heterozygous the *rs1143634*C/T* genotype appeared to be significantly higher in healthy Bashkirs (58.06%) than in healthy Tatars (32.99%, $\chi^2 = 5.4$, $p = 0.02$).

Comparative analysis of the allele and genotype frequency distributions of *rs1143634* for PUD patients and the control group demonstrated statistically significant differences in Bashkirs: the *rs1143634*C* allele was observed in 83.90% of patient chromosomes and in 64.52% of chromosomes from the control group, and the *rs1143634*C/C* genotype was detected in 71.19% in PUD individuals as compared to the relevant control group (35.48%). These variants represent markers of high risk of developing PUD ($\chi^2 = 7.61$, $p = 0.006$; OR = 2.87; 95% CI 1.40–5.86 and $\chi^2 = 9.28$, $p = 0.002$; OR = 4.49; 95% CI 1.78–11.35, respectively). Moreover, markers of decreased risk of developing PUD were revealed for ethnic Bashkirs: the rare *rs1143634*T* allele was detected in 16.10% of patients, compared to 35.48% in the control group ($\chi^2 = 7.61$, $p = 0.006$; OR = 0.35; 95% CI 0.17–0.71) and the heterozygous *rs1143634*C/T* genotype was observed in 25.42% of PUD patients and in 58.06% of healthy donors of the same ethnicity.

Individuals with duodenal ulcer and without gastric ulcer were isolated in a separate group. Comparison of this group with the combined group of healthy donors revealed that the *rs1143634*C* allele of the *IL1B* gene detected in 77.64% of patients, versus 70.4% of the control group ($\chi^2 = 6.17$, $p = 0.01$; OR = 1.46; 95% CI 1.08–1.97), and the *rs1143634*C/C* genotype observed in 61.81% of patients and in 49.63% of control individuals ($\chi^2 = 6.88$, $p = 0.009$; OR = 1.64; 95% CI 1.13–2.38) also appear to be markers of high risk of duodenal mucous membrane disease. The control group was characterized by a higher frequency of the *rs1143634*T* allele (29.60%) and C/T genotype (41.54%) as compared to the described patient group (22.36% and 31.66%, respectively)—$\chi^2 = 6.17$, $p = 0.01$; OR = 0.69; 95% CI 0.51–0.92 and $\chi^2 = 4.80$, $p = 0.03$; OR = 0.65; 95% CI 0.44–0.96, respectively.

Patients with gastric and duodenal ulcer infected with *H. pylori* on the date of blood collection were also included in the separate group. According to the comparison of allele and genotype frequency distributions of the same DNA locus between the patients and control group, no statistically significant differences were observed.

The frequencies of the *IL1RN VNTR* polymorphism (*rs71941886*) genotypes in PUD patients and control individuals from RB are shown in Table 3. Four allelic variants and five different genotypes have been previously established in the samples from our region; the distribution was shown to be similar in the

Table 3. Distribution of genotype frequencies of *rs71941886* polymorphism of the *IL1RN* gene in patients with PUD and healthy donors

Sample		Genotypes					*N*
		11	*12*	*13*	*14*	*22*	
		n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	
Patients with PUD	Russians	31 (50.0)	24 (38.71)	2 (3.23)	0	5 (8.06)	62
	Tatars	54 (55.67)	27 (27.84)	3 (3.09)	0	13 (13.40)	97
	Bashkirs	35 (62.50)	1 (33.93)	2 (3.57)	0	0	56
	Duodenal ulcer	110 (54.46)	69 (34.16)	6 (2.96)	0	17 (8.42)	202
	H. pylori	61 (54.46)	34 (30.36)	4 (3.57)	0	13 (11.61)	112
	In total	135 (55.33)	80 (32.78)	6 (2.46)	0	23 (9.43)	244
Control	Russians	61 (45.52)	57 (42.54)	6 (4.48)	2 (1.49)	8 (5.97)	134
	Tatars	49 (49.00)	39 (39.00)	0	3 (3.00)	9 (9.00)	100
	Bashkirs	20 (66.67)	7 (23.33)	1 (3.33)	0	2 (6.67)	30
	In total	136 (49.10)	110 (39.70)	8 (2.89)	5 (1.81)	18 (6.50)	277

studied groups ($p > 0.05$). The more frequent *rs71941886*1* allele was detected in patients from different ethnic groups (70.97–79.46%) and in healthy donors (69.77–80.00%). The *rs71941886*3* alelle was rare (0–1.61% in patients, 0–2.24% in the control group), and the *rs71941886*4* allele was detected only in healthy individuals with a frequency of less than 2%. Homozygous *rs71941886*1/1* genotype (50.00–62.50% in patients, 45.52–66.67% in control group) and *1/2* genotype (27.84–38.71% in patients, 23.33–42.54% in control group) appeared to be more frequent. No statistically significant associations of *rs71941886* polymorphism of the *IL1RN* gene with a risk of developing PUD were demonstrated on the basis of a comparison between PUD patients and healthy donors in accordance with their ethnicity and clinical characteristics.

The results of analysis of the allele and genotype frequency distributions of the *–251T>A* (*rs4073*) marker of the *IL8* gene are demonstrated in Table 4. All groups were characterized by an increased frequency of the *rs4073*T* allele (54.17–67.50% in patient groups with different ethnicity and 52.04–62.90% in corresponding control groups) and the *rs4073*T/A* genotype (48.34–55.00% in patients, 44.90–57.03% in healthy donors).

Sample division into subgroups in accordance with ethnical background revealed no associations of this DNA locus with a risk for developing PUD. A comparative analysis of *rs4073* conducted between patients with duodenal ulcer and control group also demonstrated no statistically significant differences ($p > 0.05$).

According to the results of comparative analysis of the allele and genotype frequency distributions of *rs4073* in the *IL8* gene between patients infected with *H. pylori* and the combined control group, the homozygous *rs4073*A/A* genotype was significantly higher observed in healthy individuals (19.39%) as compared to patients with *H. pylori* infection (10.0%)—$\chi^2 = 5.29$, $p = 0.02$; OR = 0.46; 95% CI 0.24–0.90. Moreover, a tendency was observed toward an increased frequency of the *rs4073*T* allele (62.50% in PUD patients and 54.94% in the control group) and, hence, toward a decreased frequency in the *rs4073*A* allele (37.50% in PUD patients and 45.06% in the control group , $p = 0.05$) among patients with PUD relative to individuals without pathology in gastrointestinal tract.

The allele and genotype frequency distributions of *–627C>A* (*rs1800872*) polymorphism in the *IL10* gene for PUD patients and healthy donors from RB are shown in Table 5. The most frequently observed, the *rs1800872*C* allele, was detected in 58.18–75.41% of patients with different ethnicity and in 64.89–75.00% in the control group. A similar frequency was detected for the *rs1800872*C/C* and *C/A* genotype, while the *rs1800872*A/A* genotype was rare (in total, its frequency was 4.08–6.56% in patients and 4.17–12.77% in the control group).

Differences in the allele and genotype frequency distributions of this locus between PUD patients and healthy donors of Russian, Tatar, and Bashkir ethnicity remained statistically nonsignificant ($p > 0.05$). No association of *rs1800872* of the *IL10* gene with a risk of duodenal ulcer or PUD development in the presence of *H. pylori* infection was revealed.

Table 4. Distribution of allele and genotype frequencies of *rs4073* polymorphism of the *IL8* gene in patients with PUD and healthy donors

Sample		Genotypes			Alleles		*N*
		TT	*TA*	*AA*	*T*	*A*	
		n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	
Patients with PUD	Russians	16 (26.67)	33 (55.00)	11 (18.33)	65 (54.17)	55 (45.83)	60
	Tatars	32 (30.77)	56 (53.85)	16 (15.38)	120 (57.69)	88 (42.31)	104
	Bashkirs	26 (43.33)	29 (48.34)	5 (8.33)	81 (67.50)	39 (32.50)	60
	Duodenal ulcer	70 (33.18)	112 (53.08)	29 (13.74)	252 (59.72)	170 (40.28)	211
	H. pylori	42 (35.00)	66 (55.00)	12 (10.00)	150 (62.50)	90 (37.50)	120
	In total	82 (32.42)	136 (53.75)	35 (13.83)	300 (59.29)	206 (40.71)	253
Control	Russians	33 (27.27)	69 (57.03)	19 (15.70)	135 (55.79)	107 (44.21)	121
	Tatars	29 (29.59)	44 (44.90)	25 (25.51)	102 (52.04)	94 (47.96)	98
	Bashkirs	11 (35.48)	17 (54.84)	3 (9.68)	39 (62.90)	23 (37.10)	31
	In total	77 (29.28)	135 (51.33)	51 (19.39)	289 (54.94)	237 (45.06)	263

Table 5. Distribution of allele and genotype frequencies of *rs1800872* polymorphism of the *IL10* gene in patients with PUD and healthy donors

Sample		Genotypes			Alleles		*N*
		CC	*CA*	*AA*	*C*	*A*	
		n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	
Patients with PUD	Russians	35 (57.38)	22 (36.06)	4 (6.56)	92 (75.41)	30 (24.59)	61
	Tatars	46 (46.94)	48 (48.98)	4 (4.08)	140 (71.43)	56 (28.57)	98
	Bashkirs	16 (46.96)	32 (46.52)	7 (6.52)	64 (58.18)	46 (41.82)	55
	Duodenal ulcer	91 (45.27)	96 (47.76)	14 (6.97)	278 (69.15)	124 (30.85)	201
	H. pylori	50 (43.86)	56 (49.12)	8 (7.02)	156 (68.42)	72 (31.58)	114
	In total	111 (46.96)	115 (46.52)	18 (6.52)	337 (69.06)	151 (30.94)	244
Control	Russians	67 (55.83)	46 (38.34)	7 (5.83)	180 (75.00)	60 (25.00)	120
	Tatars	40 (42.55)	42 (44.68)	12 (12.77)	122 (64.89)	66 (35.11)	94
	Bashkirs	13 (54.17)	10 (41.66)	1 (4.17)	36 (75.00)	12 (25.00)	24
	In total	128 (51.41)	101 (40.56)	20 (8.03)	357 (71.69)	141 (28.31)	249

The distribution of allele and genotype frequencies of *–308G>A* (*rs1800629*) polymorphism located in the promoter region of the *TNFA* gene is shown in Table 6. The highest frequency was observed for the *rs1800629*G* allele (83.14–88.75% in PUD patients with different ethnicity and 85.86–90.48% in the control group) and for the *G/G* genotype (67.44–80.00% in patients, 73.74–80.95% in control group). However, homozygosis for the rare allele *rs1800629*A/A* genotype was observed in less than 3% of cases. Analysis of the allele and genotype frequency distributions demonstrated the absence of statistically significant differences between the studied PUD samples and control individuals.

A meta-analysis of the studied polymorphisms of cytokine genes in Russians, Tatars, and Bashkirs was conducted; the results are shown in Table 7. A moderate level of sample heterogeneity $I^2 = 50.2\%$ (95% CI 0.0–85.6%)

Table 6. Distribution of allele and genotype frequencies of *rs1800629* polymorphism of the *TNFA* gene in patients with PUD and healthy donors

Sample		Genotypes			Alleles		*N*
		GG	*GA*	*AA*	*G*	*A*	
		n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	
Patients with PUD	Russians	43 (72.88)	16 (27.12)	0	102 (86.44)	16 (13.56)	59
	Tatars	58 (67.44)	27 (31.40)	1 (1.16)	143 (83.14)	29 (16.86)	86
	Bashkirs	32 (80.00)	7 (17.50)	1 (2.50)	71 (88.75)	9 (11.25)	40
	Duodenal ulcer	128 (72.73)	45 (25.57)	3 (1.70)	301 (85.51)	51 (14.49)	176
	H. pylori	70 (70.71)	29 (29.29)	0	169 (85.35)	29 (14.65)	99
	In total	153 (72.51)	55 (26.07)	3 (1.42)	361 (85.55)	61 (14.45)	211
Control	Russians	102 (80.95)	24 (19.05)	0	228 (90.48)	24 (9.52)	126
	Tatars	73 (73.74)	24 (24.24)	2 (2.02)	170 (85.86)	28 (14.14)	99
	Bashkirs	19 (79.17)	5 (20.83)	0	43 (89.58)	5 (10.42)	24
	In total	204 (77.86)	56 (21.38)	2 (0.76)	464 (88.55)	60 (11.45)	262

Table 7. Results of meta-analysis of the studied polymorphisms in patients with peptic ulcer disease and control individuals of Russian, Tatar, and Bashkir ethnicity

Gene	SNP	Alleles	Model with fixed effect		Model with random effect		I^2, %
			P	OR	*P*(R)	OR(R)	
IL1B	*rs1143634*	*C*	0.012	1.49	0.047	1.57	50.2
		T	0.012	0.67	0.047	0.64	50.2
IL1RN	*rs71941886*	*1*	0.83	–	–	–	0
		2	0.83	–	–	–	0
		3	–	–	0.93	–	61.5
		4	–	–	–	–	–
IL8	*rs4073*	*T*	0.43	–	–	–	0
		A	0.43	–	–	–	0
IL10	*rs1800872*	*C*	0.29	–	–	–	0
		A	0.29	–	–	–	0
TNFA	*rs1800629*	*G*	0.25	–	–	–	0
		A	0.25	–	–	–	0

P, level of significance; *P*(R), level of significance for the model with a fixed effect; I^2, Higgins' level of heterogeneity.

was demonstrated for *IL1B rs1143634*. Since this value of heterogeneity criterion (I^2) appears to be borderline, both the model with a fixed effect (Mantel–Haenszel statistic) and a model with random effect (Dersimonian–Laird statistic) were calculated. Both approaches revealed that the differences between the patients and control group appeared to be statistically significant. According to Mantel–Haenszel statistic, the odds ratio for the *rs1143634*C* allele (OR_C) was 1.49 ($p = 0.012$, 95% CI 1.10–2.03) and for the *rs1143634*T* allele (OR_T) it was 0.67 ($p = 0.012$, 95% CI 0.49–0.91). The Dersimonian–Laird method revealed the following values: $OR_C = 1.57$ ($p = 0.047$, 95% CI 1.01–2.44), $OR_T = 0.64$ ($p = 0.047$, 95% CI 0.41–0.99). According to the results of the meta-analysis, no statistically significant differences between

PUD patients and healthy donors were revealed for *rs71941886* of the *IL1RN* gene, *rs4073* of the *IL8* gene, *rs1800872* of the *IL10* gene, or *rs1800629* of the *TNFA* gene.

Together with the estimation of the role of distinct polymorphisms in the risk of developing PUD, the models of interaction between the studied loci of cytokines genes were modeled via the GMDR program (Generalized Multifactor-Dimensionality Reduction). We failed to detect statistically significant interactions between polymorphic variants *rs1143634* of the *IL1B* gene, *rs71941886* of the *IL1RN* gene, *rs4073* of the *IL8* gene, *rs1800872* of the *IL10* gene, or *rs1800629* of the *TNFA* gene resulting in liability to PUD.

DISCUSSION

Multiple studies devoted to the analysis of a predisposition to peptic ulcer disease demonstrated that allelic variants of cytokine gene polymorphisms might affect the expression level of corresponding genes in the case of *H. pylori* infection and in the case of ulcer formation on the gastric or duodenal mucous membrane [18, 19].

For the first time, an association study has been conducted for five cytokine gene polymorphisms, including interleukin-1β *IL1B* (*rs1143634*), interleukin 1 receptor antagonist 1 *IL1RN* (*rs71941886*), interleukin 8 *IL8* (*rs4073*), interleukin 10 *IL10* (*rs1800872*), and tumor necrosis factor α *TNFA* (*rs1800629*) with respect to the risk of PUD development. The most statistically significant findings were revealed for the single nucleotide polymorphism (SNP) in the *IL1B* gene.

Interleukin-1β represents a cytokine possessing anti-inflammatory activity that is considered to be one of the main initiators of local inflammatory and reparation processes in the gastric and duodenal mucous membrane during PUD [20–23]. Several researchers [24, 25] demonstrated that the presence of expressed inflammation in the pyloric antrum of the stomach resulted in a decrease in the ability to produce acid, which is primarily caused by the functional inhibition of parietal cells by *H. pylori* itself or, more probably, due to the action of pro-inflammatory cytokines. IL1β appears to be the one of the strongest inhibitors of acidic production [26]; chronic digestive organ disease was characterized by an increased concentration of this cytokine at the early stages of a disease [27]. Increased production of the described interleukin was observed in carriers of the *rs71941886*2* allele of the *ILRN* gene and the *rs1143634*C/C* genotype [28].

The present study revealed (Table 2) that the *rs1143634*C* alelle and the *C/C* genotype of the *IL1B* gene were markers of an increased risk for developing PUD in Bashkirs; these molecular markers appeared to be more frequent in a group of duodenal ulcer patients than in the control group. Moreover, markers of a decreased risk for developing PUD were detected in the same studied groups bearing the *rs1143634*T* allele and the *C/T* genotype. According to the results of meta-analysis, the significance of *rs1143634* polymorphism in developing PUD was confirmed.

The obtained findings are congruent with several studies. Thus, Garcia-Gonzalez et al. [23] established that the *rs1143634*C* allele of the *IL1B* gene was a marker of an increased risk of developing duodenal ulcer in patients infected with *H. pylori*. Abuzarova [29] has analyzed the distribution of allele and genotype frequencies of *rs1143634* polymorphism and reported that the *C/C* genotype was more frequent in PUD patients compared to control; however, these differences remained under the level of significance. The author revealed that helicobacter-mycoplasma infection was significantly more frequent in patients with gastric and duodenal ulcer with a combination of the *rs16944*T/*T*, *rs1143634*C/C*, *rs71941886*2/2*, and *rs1800896*A/G* genotypes consisting of the homozygous *C/C* genotype of *IL1B rs1143634.* However, the study of this locus in patients with gastrointestinal tract abnormalities from Iran reported that the *rs1143634*T/T* genotype appeared to be a risk factor for duodenal ulcer development [30].

The presence of a specific antagonist, IL1RA, which is encoded by the *IL1RN* gene (located on the long arm of chromosome 2), represents one of the mechanisms of IL1 activity regulation [31]. As was described above, allele *2* of the *VNTR* locus (*rs71941886*) of the *IL1RN* gene correlated with increased gene expression; moreover, the association of this allele with duodenal ulcer in Spain [23] was reported, while Yanovich et al. [32] revealed statistically significant differences in the frequency of the *rs71941886*2/L* genotype between patients with *H. pylori*-associated gastritis and patients with duodenal ulcer. Based on the present study, it has been established that the polymorphic *VNTR* variant of the *IL1RN* gene was not associated with PUD in RB (Table 3).

Interleukin 8 (chemotaxic factor of T cells and factor-activating neutrophils) appears to be one of the most important proinflammatory cytokines and plays a significant role in the mechanism of inflammatory response to *H. pylori* infection. It is considered to be the one of the main candidate genes for PUD development [33, 34]. The *IL8* gene encoding this cytokine was mapped on chromosome 4 (4q12-q21) [35] and is characterized by promoter polymorphism *–251T>A* (*rs4073*), having being studied in detail worldwide; however, the results remain contradictory [7, 33, 34]. The present study included an analysis of the allele and genotype frequency distributions of *rs4073* of the *IL8* gene in patients with PUD and the control group from RB. It revealed that the homozygous *rs4073*A/A* genotype was significantly more frequent in the control group as compared to patients with *H. pylori*; more-

over, an expressed trend for increased *rs4073*T* allele frequency in infected patients was observed compared to the healthy donors (Table 4). The findings revealed by our group are opposite those demonstrated by various researchers from different countries, which established an association of the *rs4073*A/A* genotype with PUD in the case of *H. pylori* infection [7, 36, 37]. Several foreign studies reported that distinct strains of *H. pylori* were able to induce expression of the *IL8* gene via activation of NF-κB and AP-1 transcription factors [38, 39], and an increased expression of the described gene was associated with the *rs4073*A* allele [4]. To interpret reported contradictions, additional research must be conducted on a larger sample of groups of *H. pylori*-infected patients from different ethnic backgrounds in our region. Yin et al. (2013) published the results of meta-analysis of eight independent studies of *IL8 rs4073* polymorphism in different countries (6 in Asians, 2 in Caucasians), which demonstrated that this SNP was associated with a risk of developing PUD only in Asians, since the *rs4073*A/A* genotype was significantly more frequent in the subgroups of individuals infected with *H. pylori* and patients with duodenal and gastric ulcers [34].

The *IL10* and *TNFA* genes are frequently studied as genes predisposing one to PUD, and the synthesis of encoded proteins in the stomach and duodenum correlated negatively with the presence of helicobacter infection [40, 41]. Interleukin 10 possesses the ability to inhibit production of proinflammatory cytokines via suppression of Th1 lymphocytes and stimulation of B and Th2 lymphocytes; hence, this cytokine demonstrates anti-inflammatory functions. An analysis of *–627C>A* (*rs1800872*) polymorphism of the *IL10* gene was performed in the present study and revealed the absence of an association of this locus with PUD liability (Table 5). Studies by other research groups indicating the relation of this SNP with the risk for PUD development are known. For instance, Kang et al. [7] reported that the *rs1800872*C/C* genotype demonstrated the role of the protective factor toward developing gastroduodenal pathology. Rad et al. [42] established that the *rs1800872*C* allele included in a distinct haplotype was associated with an increased level of *IL10* gene expression.

Tumor necrosis factor α possesses anti-inflammatory activity and the ability to inhibit acid synthesis in the gastric juice [41, 43]. According to the results of the study of *–308G>A* (*rs1800629*) polymorphism of *TNFA* gene (Table 6), no association of this locus with PUD was revealed. Zhang et al. [44] published the results of meta-analysis based on the findings from 16 studies devoted to the search for an association of polymorphic markers in the promoter region of the *TNFA* gene with a risk for developing duodenal ulcer. No statistically significant differences in the distribution of the allele and genotype frequencies of *rs1800629* was demonstrated between patients and healthy donors. Yanovich et al. [32] suggested a possible relation between the risk for developing duodenal ulcer and the presence of the *rs1800629*A* allele.

Therefore, an association study of polymorphic variants of five cytokines genes in patients with peptic ulcer disease and healthy donors from RB was performed. It has been detected that the *rs1143634*C* allele and the *C/C* genotype of the *IL1B* gene are markers of an increased risk of developing this disease, while the heterozygous *C/T* genotype is a marker of a decreased risk for developing PUD. An association of the *rs1143634*C* allele and the *C/C* genotype with duodenal ulcer was observed in a subgroup of patients with this disease. It has been shown that the homozygous *rs4073*A/A* genotype of the *IL8* gene was significantly more frequent in patients with *H. pylory* infection. *IL1RN rs71941886*, *IL10 rs1800872*, *TNFA rs1800629* polymorphisms demonstrated no association with developing PUD in patients from RB.

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