THE ROLE OF THE POLYMORPHISM rs28362491 OF THE *NFKB1* GENE IN THE GASTRIC CANCER DEVELOPMENT

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Abstract. Gastric cancer (GC) is one of the most common cancer types in the world with a high mortality rate. It is assumed that polymorphisms of the *NFKB1* gene that disrupt its expression predispose to the development of epithelial cancer, including GC. The aim of this study is to explore the association of polymorphism rs28362491 of *NFKB1* gene with the risk of GC development for individuals from the Volga-Ural region of Russia. The samples for the study were the DNA of 374 patients with GC and 365 healthy donors of various ethnicities (Russians, Tatars, Bashkirs). It was shown that in all studied groups the most common heterozygous genotype ID of the polymorphic locus rs28362491 of the *NFKB1* gene, alleles I and D occur with similar frequencies. Also, it has been established that for Tatars, allele D of the rs28362491 polymorphic locus of the *NFKB1* gene is a marker of an increased risk of developing GC, and allele I and genotype II are markers of a reduced risk of developing GC. Meta-analysis showed statistically significant differences in the distribution of allele frequencies of the polymorphic locus rs28362491 of the *NFKB1* gene between patients with cancer and controls when combining samples of Tatars and Bashkirs. We hypothesize that polymorphism rs28362491 of the *NFKB1* gene may contribute to the genetic structure of susceptibility to GC.

Keywords: polymorphism, association, *NFKB1* gene, gastric cancer.

List of Abbreviations

GC – Gastric cancer
NFKB1 – Nuclear Factor Kappa B Subunit 1
HDGC – Hereditary Diffuse Gastric Cancer
CDH1 – Cadherin 1
DNA – Deoxyribonucleic Acid
I – Incertin
D – Deletion

Introduction

Gastric cancer (GC) is a leading cause of human malignancy-related deaths, with an often late-stage diagnosis that limits therapeutic options (Torre *et al*., 2015). It was reported as the 7th most common cancer and the 6th leading cause of cancer death worldwide in 2022 (https://gco.iarc.fr/en). In Russia, according to statistics for 2022, cancer of this localization has dropped to 12th place in terms of incidence, however, mortality in the first year after diagnosis for GC patients remains very high and amounts to 41.9% (Kaprin, 2023). GC is one of the most common and dangerous cancers, which has a significant impact on the quality of life and leads to significant mortality among the population. This disease is a serious problem for medicine, requiring continuous study and improvement of methods of diagnosis, treatment and prevention (Deng *et al*., 2021). Genetic predisposition, nutritional features, alcohol consumption, smoking, Helicobacter pylori and Epstein–Barr virus – are the main factors that have a significant impact on the increased risk of developing GC (Machlowska *et al*., 2020).

It is well known that hereditary GC, the diffuse type (HDGC), is associated with heterozygous mutations in the E-cadherin gene, also known as the *CDH1* gene. The incidence of HDGC due to germline *CDH1* mutations varies from 1% to 3% (Shenoy, 2019). In our previous studies for the *CDH1* gene, we identified only benign genetic variants in the germinal DNA of patients with GC and identified one somatic

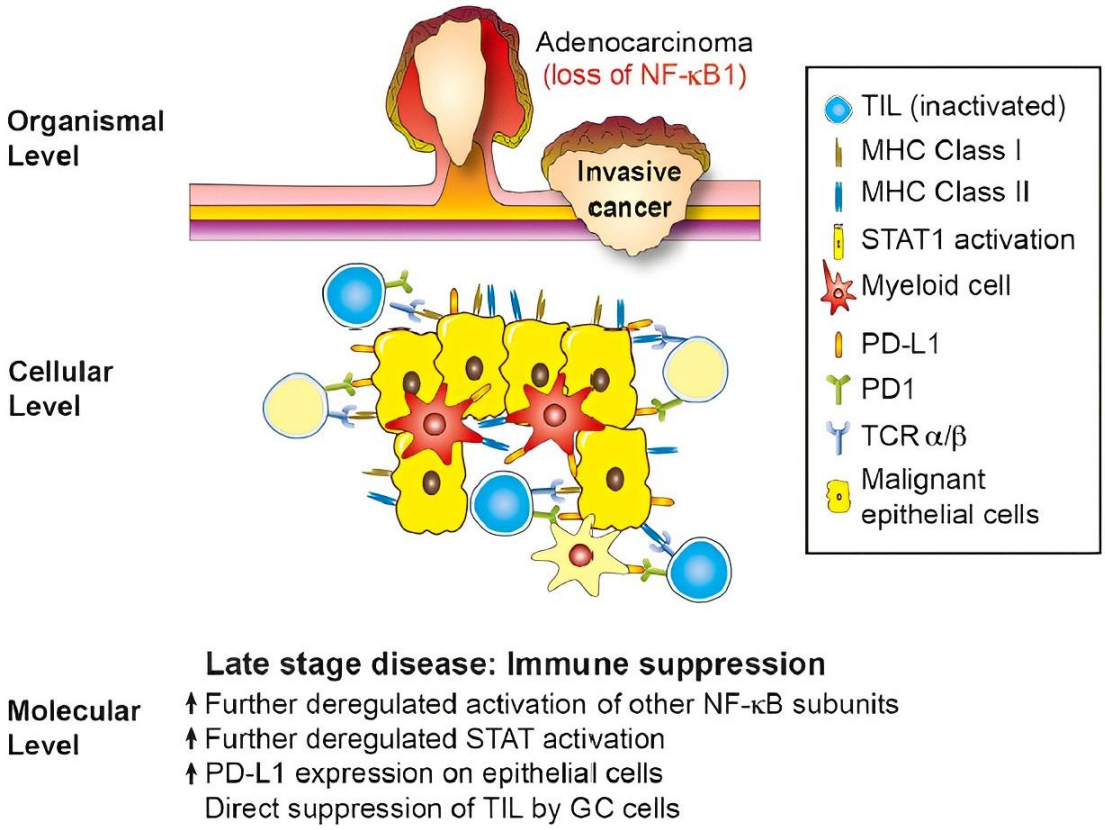

Fig. 1. Loss of NF-kB1 causes gastric cancer with aberrant inflammation and expression of immune checkpoint regulators in a STAT-1-dependent manner (O'Reilly *et al*., 2018)

mutation in one patient with a diffuse type of disease (Nurgalieva *et al*., 2023a; Nurgalieva *et al*., 2023b).

Polymorphisms in human *NFKB1* that reduce its expression have been linked to inflammatory diseases and increased risk of epithelial cancer. The links between NF-kB signaling, inflammation, and tumorigenesis are poorly understood. O'Reilly et al. reveal that NF-kB1 deficiency causes gastric cancer by dysregulating inflammation and immune checkpoints through a STAT1-dependent process. This may explain how polymorphisms in NFKB1 that impair its expression predispose to the development of epithelial cancers (Pic.1.) (O'Reilly *et al*., 2018).

Some studies have reported the association between the polymorphism, -94 ins/del ATTG (rs28362491) of *NFKB1*, and various inflammatory diseases, as well as malignant neoplasm. However, these results do not always lead to the same conclusions (Arisawa *et al*., 2012).

In this regard, we decided in this study to explore the association of polymorphism rs28362491 of *NFKB1* gene with the risk of development of GC for individuals from the Volga-Ural region of Russia.

Materials and Methods

Study populations

We used DNA samples isolated from peripheral blood of 374 patients with different ethnic origin with a histologically confirmed diagnosis of GC, who are being treated at the Republican Clinical Oncological Dispensary, Ufa, Russia in years 2017–2023. Diagnostic criteria included anamnesis data, physical examination laboratory and instrumental examinations, as well as pathological and anatomical examination data, adopted in accordance with clinical

guidelines developed jointly by the All-Russian National Union «Association of Oncologists of Russia» and the All-Russian public organization «Russian Society of Clinical Oncology». The sampling was carried out by the staff of the Surgical Department No. 1 of the Republican Clinical Oncological Dispensary in Ufa in accordance with the ethical standards of the bioethical committee, based on the Helsinki Declaration of the World Medical Association «Ethical principles for conducting scientific medical research involving a person as a subject». The age of patients ranged from 28 to 77 years, the average age of disease manifestation was 62.67 years. The share of Russians was 43%, the share of Tatars was about 44%, the share of Bashkirs in the region – 9%, and other nationalities – 3%. The gender distribution is as follows: 202 males, 172 females. Patients with GC, according to the oncology stage, were divided as follows: Stages 1 and 2 were grouped into one group (74 patients (20%)) and Stages 3 and 4 into another group (3 and 4 stages 240 patients (64%)). For the group of patients, distribution was also made depending on the histological type of GC: intestinal (36 %) and diffuse (44 %). The control group consists of 365 people: 169 Russians, 125 Tatars, 48 Bashkirs, 11 people of other nationalities. In this study, the control group was also divided by sex: the proportion of males was 59%, females – 41%.

Ethical approval

The sampling was carried out by the staff of the Surgical Department No. 1 of the Republican Clinical Oncological Dispensary in Ufa in accordance with the ethical standards of the bioethical committee developed by the WMA Declaration of Helsinki-«Ethical Principles for Medical Research Involving Human Subjects». All subjects completed a questionnaire taking into account nationality up to three generations, year of birth, smoking status, type of diet, and whether close relatives had a history of cancer. All respondents signed informed voluntary consent to participate in the study. This work was approved by the Local Ethical Committee of the Institute of Biochemistry and Genetics of the Ufa Scientific Center of the Russian Academy of Sciences (Protocol No. 14 dated 15 September 2016).

Methods

Genomic DNA was isolated from peripheral blood lymphocytes by sequential phenol-chloroform extraction (Mathew, 1984). The DNA concentration was measured using NanoDrop 2000c UV-Vis Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Genotyping of polymorphic loci was performed using the PCR-RFLP analysis. The primers used were: F: 5'-TGGG-CACAAGTCGTTATGA-3' and R: 5'- CTG-GAGCCGGTAGGGAAG-3'. Restriction was performed with enzyme Van91I (Thermo fisher scientific), the separation of fragments in 7% PAAG was as follows: allele Del – 280 bp and allele Ins – 239+45 bp. Chi-square was used to test association and Hardy–Weinberg equilibrium (HWE) for each variant. All statistical assessments were two-sided and considered to be significant when p-value was <0.05. For meta-analysis of results across samples

Russians and Tatars used the WinPepi program v. 11.32 (http://www.brixton-health.com/pepi4windows.html) (Abramson, 2011). To calculate the OR value and level of significance, models with fixed (Mantel–Haenszel method) and random (Dersimonian–Laird method) effects. To assess the statistical heterogeneity of the samples, the I^2 criterion was used (the proportion of variability due to the heterogeneity of the samples) (Higgins & Thompson, 2002). When $I^2 < 30\%$, heterogeneity was assessed as mild, when I2 was in the range of 30–50%, as moderate, and when $I^2 > 50\%$, as heterogeneous.

Results

GC patients and healthy donors from the Volga-Ural region of Russia were genotyped for a polymorphic locus rs28362491 of *NFKB1*gene. The distribution of allele and genotypes frequencies of rs28362491 depending on ethnicity performed in Tables 1 and 2.

Genotypes and alleles in GC are found in different ethnic groups in different ways: in the group of patients, the genotype ID – 48.13%,

and genotypes II and DD are found in 25% and 25.88% of cases, respectively. Among the Tatars, the genotype ID (54.82%) predominates, the genotype II occurs in 18.67% of cases, and the genotype DD in 26.51% of cases. In the Bashkir group the distribution was as follows: the most common genotype is ID (47.06%), genotype II is 23.53% and genotype DD is 29.41% of cases (Table 1).

Among healthy individuals that make up the control group, the distribution of allele frequencies and genotypes is the same. In each ethnic group, the dominant type is the genotype ID, which occurs with approximately the same frequency: Russian (48.52%), Tatar (50.40%), Bashkir (43.75%). Distribution of genotypes II and DD: Russian (23.67% and 27.81%), Tatars (31.20% and 18.40%), Bashkirs (31.25% and 25.00%) (Table 2).

Allele I and allele D both in the patient group and in the control group meet with roughly the same frequency in each ethnic group. Overall, allele I is found on 47.59% of chromosomes and allele D - 52.41% of chromosomes. In the control group, allele I is found on 51.37% of chromosomes, while allele D is found on 48.63% of chromosomes.

Among males, the most common genotype is the genotype ID, both in the patient group and in the control group (in 50.50% and 50.20% of cases, respectively). In female, the predominant genotype is ID, which occurs in 53.33% of cases and in the control group in 47.66%. Among male patients, genotypes II and DD are distributed as follows: II (23.7%), DD (25.74%), respectively. Among healthy males, genotypes II and DD occur as follows: II (27.67%), DD (22.13%), respectively. The distribution among females is as follows: GC have II – 19.77%, DD – 27.91%, healthy – II – 24.30%, DD – 28.04% of cases (Table 3).

Allele I and D in healthy males and females are observed in roughly equal proportions: male – I on 52.77% of chromosomes, D on 47.23% of chromosomes, female – I on 48.13% chromosomes, D on 51.87% of chromosomes. In patients, the picture is as follows: among males, allele I is found on 49.01% of chromosomes, allele D – on 50.99% of chromosomes, and among females, allele I is found in 45.93% of chromosomes, allele D – in 54.07% of chromosomes.

When comparing GC patients with a control group, the following was found: genotype II is less common in patients with an intestinal type GC (18.38% and 26.30%, respectively), genotype ID is slightly different (56.62% and 50.14%, respectively) and the genotype of DD is roughly equal (25% and 23.56%, respectively. Allele I (46.69% of chromosomes) is smaller in patients with an intestinal type of GC than allele D (53.31%) (Table 4).

Comparing diffuse type GC patients and control group individuals, genotype II was found to occur in roughly equal proportions (25.15% and 26.30%, respectively), heterozygous genotype ID occurs more frequently in the Control group (46.63% and 50.24%, respectively) and homozygous genotype DD, by contrast, are more common in patients with diffuse type GC (28.22% and 23.56%, respectively). Allele I is less common in patients with diffuse type GC (48.47% chromosomes, 51.37% chromosomes), and allele D, on the contrary, is more common in diffuse type than in the Control group (51.53% and 48.63%, respectively).

Analysis of the frequency distribution of alleles and genotypes of the polymorphic locus rs28362491 of the *NFKB1* gene in samples of GC patients and healthy individuals without any disorders of the gastrointestinal tract, which constitute the control group, revealed differences in Tatars. The deletion allele has been found to be a marker of increased risk of GC development (χ^2 = 5,67; P = 0.02; CI95% = = 1.09-2.11; OR = 1.50), and the allele I and genotype II are markers of reduced risk of GC development (χ^2 = 5.67; P = 0.02; CI95% = = 0.47-0.92; OR = 0.66 and χ^2 = 5.46; P = 0.02; CI95% = 0.29-0.87; OR = 0.50, respectively).

Since a risk allele was discovered for one population, it was decided to conduct a meta-analysis to understand the association's direction. When all three ethnic groups (Russians, Tatars, Bashkirs) were counted together, the heterogeneity of 50% was shown, the differences between patients and control were not true. When comparing populations in pairs, sta-

Table 1

Distribution of allele and genotype frequencies of the polymorphism rs28362491 of the *NFKB1* gene according to ethnicity among GC patients

		Genotypes						Alleles			
		II		ID		DD		I		D	
Commons	374	82	21.93%	192	51.34%	100	26.74%	356	47.59%	392	52.41%
Russians	160	40	25.00%	77	48.13%	43	26.88%	157	49.06%	163	50.94%
Tartars	166	31	18.67%	91	54.82%	44	26.51%	153	46.08%	179	53.92%
Bashkirs	34	8	23.53%	16	47.06%	10	29.41%	32	47.06%	36	52.94%
Others	12	3	25.00%	6	50.00%	3	25.00%	12	50.00%	12	50.00%

Table 2

Distribution of allele and genotype frequencies of the polymorphism rs28362491 of the *NFKB1* gene according to the ethnicity of the control group

		Genotypes						Alleles			
		II		ID		DD		I		D	
Commons	365	96	26.30%	183	50.14%	86	23.56%	375	51.37%	355	48.63%
Russians	169	40	23.67%	82	48.52%	47	27.81%	162	47.93%	176	52.07%
Tartars	125	39	31.20%	63	50.40%	23	18.40%	141	56.40%	109	43.60%
Bashkirs	48	15	31.25%	21	43.75%	12	25.00%	51	53.13%	45	46.88%
Others	11	0	0.00%	9	81.82%	2	18.18%	9	40.91%	13	59.09%

Table 3

Distribution of allele and genotype frequencies of the polymorphism rs28362491 of the *NFKB1* gene in GC patients and control groups according to sex

		Genotypes						Alleles			
		II		ID		DD		I		D	
GC	Male	48	23.76%	102	50.50%	52	25.74%	198	49.01%	206	50.99%
	Female	34	19.77%	90	52.33%	48	27.91%	158	45.93%	186	54.07%
Control	Male	70	27.67%	127	50.20%	56	22.13%	267	52.77%	239	47.23%
	Female	26	24.30%	51	47.66%	30	28.04%	103	48.13%	111	51.87%

Table 4

Distribution of allele frequencies and genotypes of the polymorphism rs28362491 of *NFKB1* gene in GC patients according to histological type

GC		Genotypes							Alleles			
			II		ID		DD		I		D	
		N	n	%	n	%	n	%	n	%	n	%
Type of GC	Intestinal	136	25	18.38	77	56.62	34	25.00	127	46.69	145	53.31
	Diffuse	163	41	25.15	76	46.63	46	28.22	158	48.47	168	51.53

Table 5

Results of a meta-analysis of polymorphism rs28362491 of *NFKB1* gene in GC patients and individuals of the control group of Russian and Tatar ethnicity

Gene	**polymorphism**	**Alleles**	**Fixed effect model**		**Random effect model**		**I^2, %**
			P	*OR*	*P(R)*	*Or(R)*	
NFKB1	rs28362491	ATTGATTG delATTG	- -	- -	0.432 0.432	- -	74.3%

Table 6

Results of meta-analysis of polymorphism rs28362491 of *NFKB1*gene in GC patients and individuals of the control group of Bashkir and Tatar ethnicity

Gene	**polymorphism**	**Alleles**	**Fixed effect model**		**Random effect model**		**I^2, %**
			P	*OR*	*P(R)*	*Or(R)*	
NFKB1	rs28362491	ATTGATTG delATTG	0.01 0.01	0.83 1.46	- -	- -	0.0%

tistically significant differences between patients and control in Tatars and Bashkirs are shown. The association of the polymorphic variant rs28362491 of the *NFKB1*gene with the risk of development of GC is shown in Tables 5, 6.

Discussion

GC is a multifactorial disease with high heterogeneity in prognosis and response to standard chemotherapy drugs. Research is relevant to elucidate the molecular mechanisms of GC development in order to identify new drugs and for prognostic purposes. NF-kB plays a potential role in the development of GC. The NF-B family of transcription factors is ubiquitously expressed and plays an important role in the regulation of a wide range of biological processes, including cell differentiation, proliferation, survival and, most importantly, immune responses and inflammation in carcinogenesis (Chaithongyot *et al.*, 2021). Dysregulation of NF-kB activation is one of the main causes of the development of GC (Dolcet *et al.*, 2005). Previously, a genomic taxonomy of GCs had been developed, which used the patterns of oncogenic pathways and NF-kB signaling has been identified as one of the dominant pathways of deregulation in GC (Ooi *et al.*, 2009). Also, a number of researchers have shown that activation of NF-kB affects gastric carcinogenesis by stimulating the activation of genes involved in cell proliferation, suppression of apoptosis, metastasis, genomic instability and drug resistance (Xu *et al.*, 2019).

We genotyped the functionally significant polymorphism rs28362491 of the *NFKB1* gene in 374 patients with stomach cancer and 365 healthy donors living in the Republic of Bashkortostan, Volga-Ural region of Russia. There is evidence that the D allele of this polymorphism is associated with the destruction of the transcription factor binding site and leads to a decrease in NF-kB expression (Karban *et al.*, 2004). Our study showed that in all studied groups, the most common heterozygous genotype ID of the rs28362491 polymorphic locus of the *NFKB1* gene, while alleles I and D occur with similar frequencies. A statistically significant association was identified only for the Tatar ethnic group. It has been established that for Tatars, allele D of the polymorphic locus rs28362491 of the *NFKB1* gene is a marker of an increased risk of GC developing, and allele I and genotype II are markers of a reduced risk of GC developing. Due to the fact that conflicting results were obtained for different ethnic groups, we decided to conduct a meta-analysis using data from the main genotyped ethnic groups: Russians, Tatars and Bashkirs. Meta-analysis of the frequency of alleles and genotypes of polymorphic locus rs28362491 gene *NFKB1* in Russian and Tatar did not give statistically significant differences between patients and control. In meta-analysis of Bashkir and Tatar samples, it was found that the allele D of the polymorphic locus rs28362491, which is a marker of increased risk of developing GC for the Tatar population, is also a high-risk marker. It has also been found that the allele I of polymorphic locus rs28362491, which is a low-risk marker for the Tatar population, is significant for the Bashkir population as a low-risk marker.

Our results are partly consistent with the work of other researchers. Arisawa T. and collegs demonstrated the important role of NF-κB in the inflammatory response to *H.pylori* colonization. The authors investigated the effect of *NFKB1* -94 ins/del (rs28362491) and -449 C>G (rs72696119) polymorphisms on aberrant gene methylation during *H.pylori* infection. They found that -94 DD homozygotes were significantly associated with the risk of developing high methylation of the CpG islands of the *DAPK* and *CDH1* genes associated with GC. In addition, the number of these methylated genes increased significantly with age in DD homozygotes infected with H. pylori, but not in infected carriers of the insertion. The rs28362491 was significantly associated with an increased risk of developing age-related gene methylation in noncancerous gastric mucosa during H. pylori inflammation (Arisawa *et al.*, 2012). Another study from Japan showed that homozygote rs28362941 DD was significantly associated with the development of GC, in particular, it was strongly as-

sociated with dif-fuse type GC. In addition, the authors showed that this polymorphism is associated with tumor progression, such as tumor invasion and lymph node metastasis. Inflammatory cell infiltration into non-cancerous gastric mucosa was higher in subjects with the rs28362491 DD genotype compared to subjects with other genotypes (Arisawa *et al.*, 2013).

Thus, we can conclude that the rs28362491 polymorphism of *NFKB1* gene may influence the development of GC and it is advisable to test it to identify predisposition to an increased risk of GC developing.

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The authors declare no conflict of interest.

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