ISSN 2304-3415, Russian Open Medical Journal
2025. Volume 14. Issue 1 (March). Article CID e0108
DOI: 10.15275/rusomj.2025.0108

Original article

The role of pathogenic germline mutations of *BRCA1/2*, *NBS1*, and *CHEK2* genes in gastric cancer development within the Volga-Ural region of Russia

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Received 15 July 2024, Revised 20 October 2024, Accepted 29 November 2024

Abstract: *Background and objective* — Gastric cancer (GC) is one of the most common cancers worldwide with a high mortality rate. Hereditary predisposition to GC is still not fully understood. The objective of this study was to compare the prevalence of mutations in the *BRCA1* (c.*68_69delAG, c.4035delA, c.5266dupC, c.3700_3704delGTAAA, c.3756_3759delGTCT, c.181T>G, c.1961delA*), *BRCA2* (c.*5946delT*), *CHEK2* (c.*1100delC, c.115+1G>A*) and *NBS1* (*c.657_661delGTTTT*) genes in patients with GC and healthy donors from the Volga-Ural region of Russia.

Methods — The material for the study was DNA samples from 415 patients with GC and 400 healthy donors. Genomic DNA was isolated from peripheral blood lymphocytes using sequential phenol-chloroform extraction. Genotyping of mutations was performed using real-time polymerase chain reaction with DNA melt curve detection.

Results — A total of 8 individuals with a heterozygous germline mutation were identified: 2 patients with GC of Bashkir and Tatar nationality had c.5266dupC in the *BRCA1* gene, 1 Tatar woman with GC had *c.3756_3759delGTCT* in the *BRCA1* gene, 2 men with GC of Russian nationality were carriers of *c.657_661delGTTTT* in the *NBS1* gene, 2 patients with GC of Tatar and Russian nationality were carriers of c.115+1G>A in the *CHEK2* gene, and 1 Tatar woman from the control group had *c.181T>G* in the *BRCA1* gene.

Conclusion — These results open up new opportunities for studying the molecular basis of GC and developing targeted treatments for patients with these mutations. Further research is needed to fully uncover the clinical potential of these findings and improve the treatment of GC in affected populations.

Keywords: gastric cancer, mutation, germline mutation, pathogenic variant.

Cite as Nurgalieva AKh, Petrova SG, Gallyamova LF, Ekomasova NV, Sakaeva DD, Fedorova YuYu, Dzhaubermezov MA, Abdeev RR, Rakhimov RR, Khusnutdinova EK, Prokofyeva DS. The role of pathogenic germline mutations of *BRCA1/2*, *NBS1*, and *CHEK2* genes in gastric cancer development within the Volga-Ural region of Russia. *Russian Open Medical Journal* 2025; 14: e0108.

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Introduction

Gastric cancer (GC) is a common disease that remains one of the leading causes of cancer death worldwide. In 2022, it was the 7th most common cancer and the 6th leading cause of cancer death worldwide (https://gco.iarc.fr/en). In Russia, according to statistics for 2022, cancer of this localization dropped to 12th place in terms of incidence, but mortality in the first year after diagnosis in patients with GC remains very high (41.9%) [1].

GC is a multifactorial disease, the development of which can be influenced by many factors, both environmental and genetic. It is noted that family history, diet, alcohol consumption, smoking, *Helicobacter pylori* and Epstein-Barr virus (EBV) are several factors that have been noted to have a significant impact on the increased risk of developing GC [2]. Family history of GC is also one of the most important risk factors. It is well known that hereditary diffuse gastric cancer (HDGC) is associated with heterozygous mutations in the E-cadherin gene, also known as the *CDH1* gene. The frequency of HDGC due to CDH1 germline mutations varies from 1% to 3% [3]. In our previous studies of the CDH1 gene, we identified solely benign genetic variants in the germline DNA of GC patients and identified one somatic mutation in one patient with HDGC [4, 5]. According to available data, approximately 8–30% of GC patients have a positive family history. However, not all of these cancers are hereditary, and the underlying genetic alteration remains unknown in 60% of cases [6]. DNA double strand breaks (DSB) are among the most serious threats to cancer cell survival and are repaired by the mechanisms of homologous recombination and non-homologous end joining. DSB repair is initiated by the combined efforts of ataxia-telangiectasia mutated (*ATM*) and a protein complex consisting of meiotic recombination encoded 11 (*MRE11*), DSB repair protein (*RAD50*), and Nijmegen breakage syndrome 1 (*NBS1*) proteins, forming the MRE11–RAD50–NBS1 (MRN) complex. In the nucleus, the MRN complex

binds to DNA DSB sites where it recruits and activates *ATM*, which can then phosphorylate several substrates including phosphorylated H2A histone family member X (γH2AX), p53 binding protein (153BP1), structural maintenance of chromosomes protein 1 (*SMC1*), breast cancer early onset 1 (*BRCA1*), and checkpoint kinase 2 (*CHK2*) to induce cell cycle arrest and apoptosis of cancer cells [7]. It was reported that the expression levels of *MRE11*, *RAD50*, and *NBS1* were higher in GC tissues than in non-cancerous tissues [8]. The association between *BRCA1*/2 germline mutation and increased risk of GC has been demonstrated in previous studies for families with hereditary breast and ovarian cancers. Regarding familial GC, a recent large-scale study showed that *BRCA2* germline mutations were identified in patients whose family history met the criteria for HDGC but who lacked CDH1 mutations. Therefore, it is possible that *BRCA1*/2 germline mutations may cause familial predisposition to GC [9]. Another gene known to be involved in the progression of various tumors is *CHEK2*. Some studies have reported that *CHEK2* is associated with GC. It has been found that aberrant expression of *CHEK2* plays a critical role in the development and progression of this pathology and that *CHEK2* is involved in GC chemotherapy by disrupting DNA damage repair [10, 11].

Genes involved in the DNA damage repair pathway (*PARP1*, *BRCA1*/2, *ATM*, *CHEK2*, *NBS1* and others) are also promising for finding driver mutations for GC. They play a critical role in tumorigenesis, progression, treatment, prognosis and other aspects of various types of cancer, including GC. The role of these genes in the development of GC and the prospects for their use for treatment, as well as the exact mechanisms of DNA damage repair in cells, should be clarified in further experiments [12]. We decided to search our samples of GC patients and healthy individuals for the most known mutations in some of the above genes, such as *BRCA1*/2, *ATM*, *CHEK2* and *NBS1*. Thus, the objective of this study was to determine the prevalence of *BRCA1* (*c.68_69delAG, c.4035delA, c.5266dupC, c.3700_3704delGTAAA, c.3756_3759delGTCT, c.181T>G, c.1961delA*), *BRCA2* (c.*5946delT*), *CHEK2* (*c.1100delC, c.115+1G>A*), and *NBS1* (c.657*_661delGTTTT*) gene mutations in GC patients from the Volga-Ural region of Russia. We also analyzed these genetic variants in stomach tumor samples and control samples from healthy donors.

Material and Methods

Study sample: patients

We used DNA samples isolated from the peripheral blood of 415 patients of various ethnicities with a histologically confirmed diagnosis of GC. All study subjects were treated at the Clinical Oncology Dispensary of the Republic of Bashkortostan Ministry of Healthcare (Ufa, Russia) in 2017-2023. In accordance with the clinical guidelines developed jointly by the Association of Russian Oncologists and Russian Society of Clinical Oncology, the diagnostic criteria included the data of the patient anamnesis, physical examination, laboratory and instrumental studies, and pathological examination. The study material was collected by the staff of the Division of Surgery No. 1 of the Republican Clinical Oncology Dispensary in accordance with the ethical standards of the bioethics committee, based on the Declaration of Helsinki by the World Medical Association, *Ethical Principles for Medical Research Involving Human Participants*. The patient age ranged from 28 to 77 years; the mean age of disease manifestation was 62.67 years. Inclusion criteria for the group of patients were as follows: histologically confirmed GC, residence in the Republic of Bashkortostan since birth, analyzed DNA of unrelated individuals, and written informed consent. A more detailed description of the samples is presented in *Table* 1.

Study sample: control subjects

A group of healthy unrelated donors without any gastrointestinal diseases, consisting of 400 people of different ethnic backgrounds, was examined as a control. Inclusion criteria for this group were: absence of any clinical and laboratory symptoms of gastrointestinal diseases and oncological diseases, no family history of gastrointestinal diseases and oncological diseases, residence in the Republic of Bashkortostan since birth, analyzed DNA of unrelated individuals and written informed consent. Patients with GC and control group subjects were similar in terms of their age, ethnicity, and gender. A more detailed description of the samples is presented in *Table* 1.

Table 1. Clinicopathological features of patients with gastric cancer and healthy donors

	Patients with gastric cancer (n=415)	*Control subjects (n=400)*
Age (mean±SE), years*	62.67±0.58	58.06 ±0.73
Ethnicity, n (%)		
Russians	177 (42.67)	169 (42.15)
Tatars	192 (46.18)	168 (42.05)
Bashkirs	38 (9.24)	49 (12.26)
Other ethnicities (Ukrainians, Chuvash, Jews, mestizos)	8 (1.91)	14 (3.54)
Gender, n (%)		
Male	238 (57.32)	237 (59.29)
Female	177 (42.68)	163 (40.71)
TNM stage of gastric cancer, n (%)		
I	23 (5.52)	-
II	75 (18.18)	-
III	279 (67.21)	-
IV	38 (9.09)	-
Well-differentiated and moderately differentiated gastric cancer	188 (45.30)	-
Poorly differentiated and undifferentiated-type gastric cancer	227 (54.70)	-

*p=0.16.

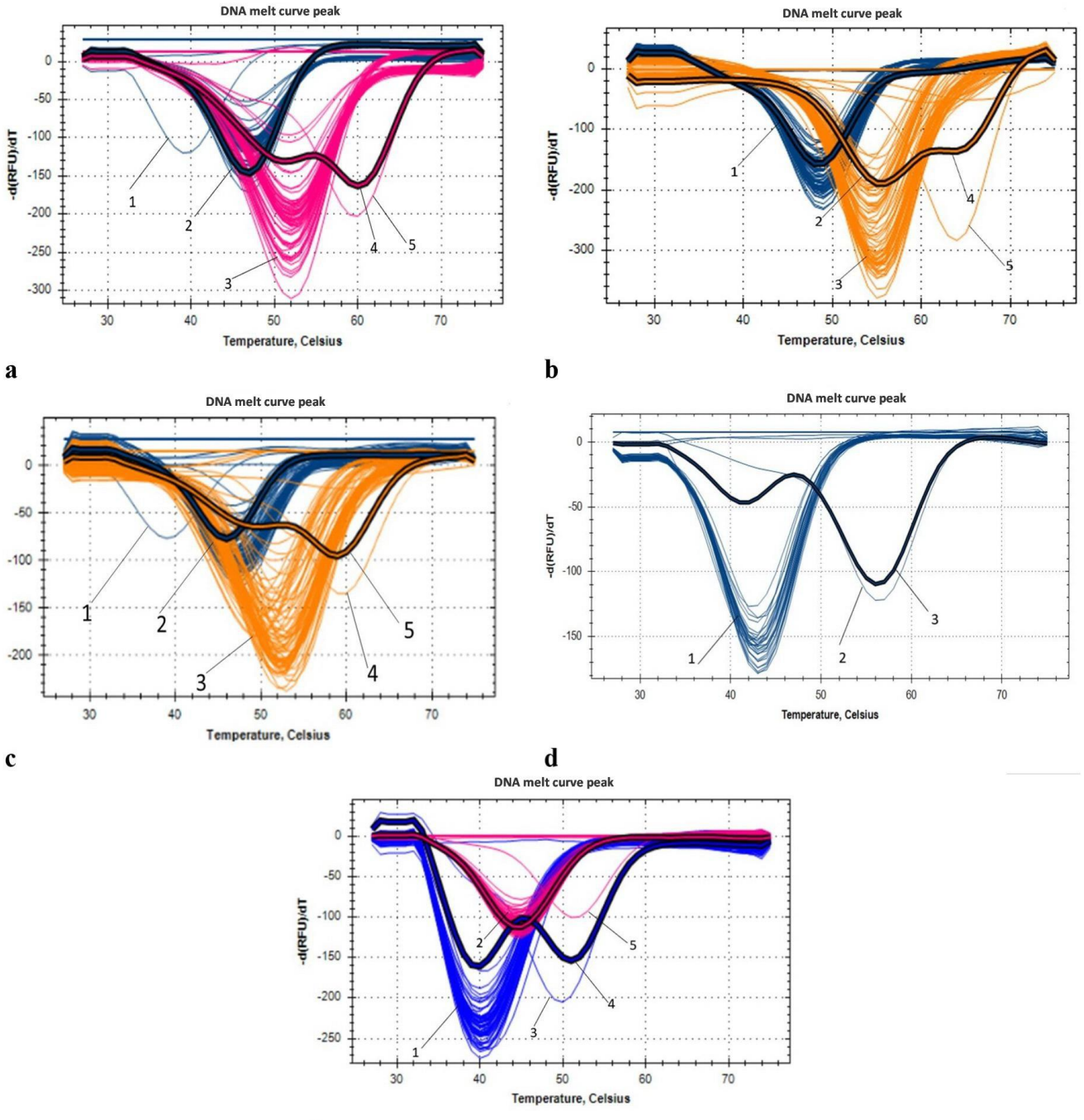

Figure 1. DNA melt curve derivatives. a. DNA melt curve graph demonstrating genotyping of a patient with gastric cancer with N/N genotype for the *c.4035delA* mutation and N/insC genotype for the c.5266dupC mutation of the *BRCA1* gene (1 – mutation in homozygous state for *c.4035delA*, 2 – normal in homozygous state for *c.4035delA*, 3 – normal in homozygous state for *c.5266dupC*, 4 – mutation in homozygous state for *c.5266dupC*, 5 – mutation in homozygous state for *c.5266dupC*); **b.** DNA melt curve graph showing genotyping of a patient with gastric cancer with N/N genotype for the *c.68_69delAG* mutation and N/delGTCT genotype for the *c.3756_3759delGTCT* mutation of the *BRCA1* gene (1 – normal in homozygous state for *c.68_69delAG*, 2 – mutation in homozygous state for *c.68_69delAG*, 3 – normal in homozygous state for *c.3756_3759delGTCT*, 4 – mutation in homozygous state for *c.3756_3759delGTCT*, 5 – mutation in homozygous state for *c.3756_3759delGTCT*); **c.** DNA melt curve graph showing genotyping of a patient with gastric cancer with N/N genotype for the *c.3700_3704delGTAAA* mutation and T/G genotype for the *c.181T>G* mutation of the *BRCA1* gene (1 – mutation in homozygous state for *c.3700_3704delGTAAA*, 2 – normal in homozygous state for *c.3700_3704delGTAAA*, 3 – normal in homozygous state for *c.181T>G*, 4 – mutation in homozygous state for c.181T>G, 5 – mutation in homozygous state for c.181T>G); **d.** DNA melt curve graph demonstrating genotyping of a patient with gastric cancer with the N/delGTTTT genotype for the *c.657_661delGTTTT* mutation of the *NBS1* gene (1 – normal in homozygous state for *c.657_661delGTTTT*, 2 – mutation in homozygous state for *c.657_661delGTTTT*, 3 – mutation in homozygous state for *c.657_661delGTTTT*); **e.** DNA melt curve plot showing genotyping of a patient with gastric cancer with N/N genotype for the *c.1100delC mutation* and G/A genotype for the *c.115+1G>A* mutation of the *CHEK2* gene (1 – normal in homozygous state for *c.115+1G>A*, 2 – normal in homozygous state for *c.1100delC*, 3 – mutation in homozygous state for *c.115+1G>A*, 4 – mutation in homozygous state for *c.115+1G>A*, 5 – mutation in homozygous state for *c.1100delC*).

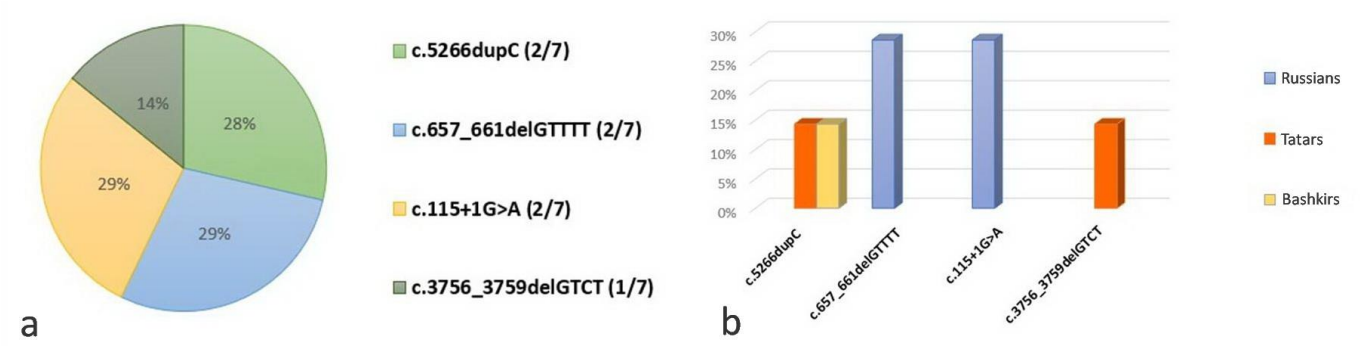

Figure 2. Spectrum of identified mutations in the *BRCA1*, *NBS1*, *CHEK2* genes: (a) mutation ratio; (b) ethnicity-based spectrum and frequency of mutations in patients with gastric cancer.

Genotyping

Genomic DNA was isolated from peripheral blood lymphocytes using the method of sequential phenol-chloroform extraction [13]. A total of 415 patients with GC and 400 healthy donors were tested for mutations in the following genes: *BRCA1* [*c.68_69delAG* (*185delAG*), *c.4035delA* (*4153delA*), *c.5266dupC* (*5382insC*), *c.3700_3704delGTAAA* (*3819del5*), *c.3756_3759delGTCT* (*3875del4*), *c.181T>G* (*T300G*), *c.1961delA* (*2080delA*)], *BRCA2* [*c.5946delT* (*6174delT*)], *CHEK2* [*c.1100delC* (*1100delC*), *c.115+1G>A* (*IVS2+1G>A)*] and *NBS1* [*c.657_661delGTTTT* (*657del5*)].

Genotyping of mutations was carried out by the method of real-time polymerase chain reaction with detection of DNA melt curves using the following test kits: RealBest-Genetics *BRCA1 185delAG/3875del4*, RealBest-Genetics *BRCA1 4153delA/5382insC*, RealBest-Genetics *BRCA1 3819del5/T300G*, RealBest-Genetics *BRCA1 2080delA(insA)/BRCA2 6174delT*, RealBest-Genetics *NBS1* and RealBest-Genetics *CHEK2* (Vector Best, Russia, https://vector-best.ru/catalog/ptsr/nabory/odnonukleotidnye-polimorfizmy-i-mutatsii-v-genakh-cheloveka/). PCR was performed using CFX96 (Bio-Rad, USA). Each set included the following control samples: *normal homozygous* and *mutant homozygous*.

Statistical data processing

Statistical analysis was performed using Statistica v. 6.0 software (StatSoft Inc., Tulsa, OK, USA). Clinical characteristics of the subjects are presented as mean ± SD. Comparisons between the two groups were performed using the Mann-Whitney U test for data characterized by non-normal distribution.

Results

All patients with GC (n=415) and healthy donors (n=400) were genotyped for 11 mutations: 7 in the *BRCA1* gene (*c.68_69delAG, c.4035delA, c.5266dupC, c.3700_3704delGTAAA, c.3756_3759delGTCT, c.181T>G, c.1961delA*), 1 (*c.5946delT*) in the *BRCA2* gene, 2 (*c.1100delC, c.115+1G>A*) in the *CHEK2* gene and 1 (*c.657_661delGTTTT*) in the *NBS1* gene. A total of 8 individuals with a germline mutation in heterozygous condition were identified: 2 patients with GC of Bashkir and Tatar nationality had *c.5266dupC* in the *BRCA1* gene, 1 Tatar woman with GC had *c.3756_3759delGTCT* in the *BRCA1* gene, 2 men with GC of Russian nationality were carriers of *c.657_661delGTTTT* in the *NBS1* gene, 2 patients with GC of Tatar and Russian nationalities were carriers of c.115+1G>A in the *CHEK2* gene, and 1 woman of Tatar nationality from the control group had *c.181T>G* in the *BRCA1* gene. Examples of DNA melt curve graphs for samples with identified mutations are shown in *Figure* 1.

We also identified the *c.115+1G>A* splice site mutation in the *CHEK2* gene in 2 patients with GC. Patient #6 was a 78-year-old man with poorly differentiated gastric adenocarcinoma with mucus formation, patient #7 was a 66-year-old woman with moderately differentiated gastric adenocarcinoma. Both patients were also of Russian nationality. There was evidence that the father of patient #7 suffered from melanoma and his aunt suffered from uterine cancer. Thus, the prevalence of the *c.115+1G>A* mutation in the *CHEK2* gene among patients with GC in total sample was 0.48% (2/415), while in people of Russian nationality it amounted to 1.13% (2/177). The *c.1100delC* genetic variant in the *CHEK2* gene was not detected in either patients or healthy donors examined by us in the course of our study. The spectrum of identified mutations in the *BRCA1*, *NBS1*, and *CHEK2* genes is presented in *Figure* 2.

Discussion

We screened patients with GC (n=415) and healthy donors (n=400) from the Volga-Ural region of Russia for 11 known cancer-predisposing mutations in the *BRCA1* gene (*c.68_69delAG, c.4035delA, c.5266dupC, c.3700_3704delGTAAA, c.3756_3759delGTCT, c.181T>G, c.1961delA*), *BRCA2* gene (*c.5946delT*), *CHEK2* gene (*c.1100delC, c.115+1G>A*) and *NBS1* gene (*c.657_661delGTTTT*). The main part of both samples (cases and control subjects) were the most common ethnic groups in our region: Russians, Tatars and Bashkirs.

Pathogenic *BRCA1*/2 variants are widely recognized as major risk factors mainly for breast and ovarian cancer, while their role in gastrointestinal malignancies remains unclear. The prevalence of *BRCA1* and *BRCA2* mutations in GC varies among different populations and geographic regions. *BRCA1*/2 are tumor suppressor genes involved in pathways important for DNA damage control, such as recognition, transcriptional regulation, and repair of DNA DSB; and such functions are vital for all cell types to avoid mutation development. *BRCA1*/2 mutations have been reported to cause the sixfold increase in lifetime risk of GC among first-degree relatives of *BRCA1*/2 mutation carriers [14]. In our study, out of seven tested mutations in the *BRCA1* gene, we identified two in GC patients and one in a healthy donor. Mutation *c.5266dupC* (*p.Gln1756fs*) is one of the most common in cancer. We confirmed

that the prevalence of *c.5266dupC* mutation among all examined patients with GC was 0.48%, and when divided into subgroups depending on ethnicity, the mutation frequency was 2.6% among Bashkirs and 0.52% among Tatars. Of course, it should be noted that there were only 38 Bashkirs in our sample. Hence, this frequency was perhaps due to the sample size. However, these numbers reflect the actual proportions of ethnic groups in our region. Consequently, the study must be continued and the sample of Bashkirs must be increased for a more accurate understanding of the effect of this mutation on GC. It is also interesting that among Bashkirs of our region, solely *c.5266dupC* mutation was detected among other malignant neoplasms (prostate cancer, ovarian cancer) [15, 16]. Our study did not detect other mutations we were looking for in Bashkirs. We also discovered the *c.3756_3759delGTCT* (*p.Ser1253Argfs*) mutation among all patients with GC (0.24%) and among Tatars (0.52%). One of the mutations in the *BRCA1* gene (*c.181T>G*) was found only in a healthy woman of Tatar nationality (its prevalence was 0.25% in the total sample and 0.60%in Tatars). In our study, the *c.5946delT* mutation in the *BRCA2* gene was not revealed in patients with GC. In general, this genetic variant was not typical for the populations of our region and was not found in other oncological diseases [15, 16]. In 2016, a Polish study was conducted on the frequency of mutations in the *BRCA1/2* genes in patients with GC. All patients were genotyped for three Polish *BRCA1* founder mutations (*5382insC, C61G, and 4153delA*) and nine known recurrent mutations in the *BRCA1* and *BRCA2* genes. Among the tested mutations, the authors identified only one *BRCA1* founder mutation, *5382insC* (*c.5266dupC*), which was detected in 2 (0.63%) of 317 cases of GC [17]. Earlier, in an Israeli study, selected *BRCA2* mutations were detected in 5.7% of 35 patients with GC [18]. In a recent study by Ichikawa H. et al., *BRCA1/2* germline mutations were identified in three Japanese patients with GC who had a familial component [9]. Yaz et al. published the results of a 2023 study in which they investigated *BRCA1* and *BRCA2* mutations in Pakistani breast cancer patients and their functional consequences using next-generation sequencing. Pathogenic mutations previously not documented in this context were identified by the authors. Expression analyses of *BRCA1* and *BRCA2* genes at both the mRNA and protein levels revealed consistent downregulation in breast cancer samples with pathogenic mutations [19].

Mutations in the homozygous state of the *NBS1* gene are responsible for a rare disorder (NBS; OMIM 251260), a rare autosomal recessive disorder characterized by chromosomal instability and hypersensitivity to ionizing radiation. Epidemiological data suggest that the *NBS1* gene can be considered a cancer susceptibility factor, as evidenced by the fact that nearly 40% of patients with NBS develop a malignancy before reaching the age of 21 years. *NBS1* heterozygotes, who are clinically asymptomatic, are known to exhibit an increased risk of developing certain types of malignancies [20]. The best-known mutation in the *NBS1* gene is *c.657_661del* (*delGTTTT*) (*p.Lys219fs*), also known as *657del5*. Our study showed that *c.657_661delGTTTT* occurs in patients with GC in our region with a frequency of 0.48% of the total sample, but this mutation was found only in Russians (1.13%; 2 patients with GC). In 2004 study by Steffen J. in Poland, the frequencies of the *657del5* germline mutation were assessed in 1,683 patients with various malignant tumors. The authors noted that in two carriers of the *657del5* mutation with non-Hodgkin lymphoma, these malignancies developed in extranodal sites (one in the stomach and the other on the soft palate), which are rare localizations for such tumors [21]. While the *657del5* mutation of the *NBS1* gene has been reported to predispose to common cancer types almost exclusively in Slavic populations, the first identification in a Japanese population of an unprecedented type of heterozygous *NBS1* mutant, named *IVS11+2insT*, has been reported by Ebi H. et al. [22] who identified this mutation in the *NBS1* gene in 2% (2 of 96) of heterozygous individuals with GC.

CHEK2 is a tumor suppressor gene. Its functions play a central role in the induction of cell cycle arrest and apoptosis following DNA damage. Germline mutations in this gene have been reported in families of patients with cancer; in particular, in breast and prostate cancer, as well as in other cancer types, including GC. Machlowska J. et al. showed that nuclear expression of *CHEK2* was high in all GC subtypes, but the prevalence of cytoplasmic *CHEK2* expression and nuclear *p-CHEK2* expression was significantly higher in conventional GC vs. early-onset GC tissues [23]. In our study, we identified a splice site mutation, *c.115+1G>A* (*IVS2+1G>A*), in the *CHEK2* gene in two Russian patients with GC (the prevalence of this mutation was 0.48% in the total sample, and 1.13% in individuals of Russian nationality). However, we did not detect the *c.1100delC* genetic variant in the *CHEK2* gene in either the examined patients or healthy donors. Teodorczyk U. et al. established that the *c.115+1G>A* mutation in the *CHEK2* gene was strongly associated with GC in the Polish population [24]. It was shown that the prevalence of the *c.115+1G>A* germline mutation was 1% and that of the *c.1100delC* germline mutation was 3% in Chinese GC patients. The authors also found that overexpression of the *CHEK2* mutation suppressed the expression of E-cadherin and increased the expression of vimentin, thereby indicating the mechanism underlying the altered biological behavior. These results suggest that there is a correlation between the *CHEK2* gene mutation and GC [25]. A case-control study in China demonstrated that four single-nucleotide polymorphisms (SNPs: *rs201688553*, *rs376099090*, *rs777046932*, and *rs372452522*) in *CHEK2 1100delC* achieved significant difference in their distribution between GC cases and control subjects. Moreover, one polymorphism (*rs7289973*) and a novel genetic variant (*IVS2-372T>C*) in *CHEK2 IVS2+1G>A* locations were identified, which showed significant difference in their distribution between GC cases and control individuals [26].

Conclusion

Current data on the populations of the Volga-Ural region of Russia indicate a low frequency of the studied mutations among patients with GC. However, it should be noted that some mutations were identified only in representatives of certain ethnic groups (Russians, Tatars and Bashkirs). It is possible that for them, the studied germline genetic variants may be associated with a high risk of developing GC. Genetic testing of *BRCA1*/2, *NBS1*, and *CHEK2* in patients with GC, especially with a familial component, can help optimize medical care, including cancer surveillance and the choice of treatment methods in the era of precision medicine.

Limitations

Limitations of the data collected in our study from a therapeutic perspective were not analyzed.

Ethical approval

The study was approved by the Ethics Committee of the Institute of Biochemistry and Genetics, Ufa Federal Research Center of the Russian Academy of Sciences (Protocol No. 14 of September 15, 2016). Written informed consent was obtained from each participant in accordance with the Declaration of Helsinki outlining the principles of medical research involving human subjects.

Acknowledgments

Our study was supported by the Government Procurement No. № 075-03-2024-123/1 from the Russian Federation Ministry of Science and Higher Education and the Grant from the Republic of Bashkortostan to young scientists (Agreement No. 1 of August 14, 2023).

Conflict of interest

The authors declare no conflicts of interest.

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