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Spectrum and frequency of BRCA1, BRCA2, CHEK2, PALB2, RAD50 mutations in breast cancer patients in the Republic of Bashkortostan

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Abstract

Aim. To assess the spectrum and frequency of mutations in patients with hereditary breast cancer from the Republic of Bashkortostan.

Methods. The material for the study was sections of fresh frozen, formalin-fixed and paraffin-embedded tumor tissue from 100 unrelated patients with a histologically confirmed diagnosis of breast cancer. The study was carried out using two methods: real-time polymerase chain reaction and next-generation sequencing-NGS.

Results. By using real-time polymerase chain reaction (PCR), the 5382insC mutation in the BRCA1 gene was detected in 12 cases. By using a next-generation sequencing method (NGS), highly penetrant mutations in BRCA1, BRCA2, CHEK2, PALB2 and RAD50 were revealed in 16 patients. In total, these methods detected mutations in 28 patients. Out of a total of probands in the BRCA1 gene, mutations were detected in 13 patients, that included 12 patients with the 5382insC mutation, and 1 patient with c.3143delG. In the BRCA2 gene, mutations were revealed in 3 patients, of which c.6621_6622del in 2 patients and c.-39-1_-39delGA in 1 patient. Mutations in CHEK2 were detected in 5 patients: c.470T>C in 3 patients, c.444+1G>A in 2 patients. The 1592delT mutation in PALB2 was found in 4 patients. The c.2157delA mutation in RAD50 was detected in 3 patients.

Conclusion. Pathogenic mutations in BRCA1/2, CHEK2, PALB2 and RAD50 were found in 28 patients with a hereditary feature of the disease; the identification of highly penetrant mutations in probands allowed us to determine their relatives, probable carriers of mutations, which were referred for genetic counselling.

Keywords: breast cancer, diagnostics, mutation, genes BRCA1, BRCA2, CHEK2, PALB2, RAD50.

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Background. Breast cancer (BC) is a complex heterogeneous disease defined by the presence or absence of overexpression of receptors on the surface of tumor cells that correlate with the presence of penetrant mutations. In the Republic of Bashkortostan (RB), as in the Russian Federation (RF), BC occupies a leading position among women's malignant neoplasm (up to 29%) and death cause [1].

It is known that 5–10% of all cases of BC have a hereditary component [2,3], of which 20–50% are caused by germinal mutations in the predisposition genes of BC with high penetrance—*BRCA1* and *BRCA2*. In addition, in BC, mutations in other genes responsible for genome integrity and epigenetic changes at the deoxyribonucleic acid (DNA) level are detected [2,4].

Hereditary forms of malignant neoplasms occupy a special position due to their frequent development at a young age, increased metastasis risk, unfavorable prognosis factors, and limited treatment options. Primary multiple malignancies are often associated with hereditary syndromes. Also, the presence of BRCA mutations increases the risk of developing BC on the opposite side and ovarian cancer (OC) [2,5].

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The *BRCA1* gene is located on the long arm of chromosome 17 (locus 17q21); normally, the protein encoded by it suppresses signal transmission of the estrogen receptors of the breast epithelium. Gene mutations are inherited by an autosomal dominant type with incomplete penetrance and are associated with BC. More often, these tumors do not contain estrogen receptors. Carriers of BRCA1 mutations have a 50–85% risk of BC and 15–45% risk of OC. The *BRCA2* gene is located on the long arm of chromosome 13 (13q12-13), and its mutations are associated with up to 20% of cases of familial BC [3,4,6].

Nowadays, driver mutations in BC have been established. For oncologists, it is important to identify the most studied mutations of the *BRCA1/2* genes for patients with BC and OC as they are an integral part of the choice of treatment tactics. The presence of BRCA1/2 mutations makes the tumor sensitive to PARP inhibitors, and in preclinical studies, this was associated with the persistence of DNA damage, which is normally corrected by homologous repair [7]. A subsequent clinical phase 3 OLYMPIAD trial [8] confirmed the efficacy of PARP inhibitors in the presence of BRCA1/2 mutations.

The information obtained about the driver mutations of patients allows us to assess both the epidemiological situation of the population and the genetic risk of developing a malignant neoplasm of proband's relatives who sought advice. The correct genealogy collection and analysis, followed by mutation identification and genetic counseling, make it possible to ensure timely measures for the diagnosis of cancer risk and its prevention in families burdened by a hereditary predisposition to cancer.

In RB, when a patient is admitted to the Republican Medical Genetic Center, the hereditary nature of malignant diseases may be determined while taking medical history with the use of a clinical-genealogical method. Subsequently, in the groups of patients with BC and OC, a category of high-risk patients is identified, indicating the hereditary nature of the disease. In accordance with the algorithm of molecular genetic testing for determining hereditary BC, the age of the disease manifestation, the molecular subtype of BC, a burdened family history, and the development of primary multiple forms of malignant neoplasm are considered [9].

If there are signs of hereditary malignancy, a polymerase chain reaction (PCR) is used to determine the most common germinal mutations in the *BRCA1/2* genes in the European population in real time.

In the next stage, the "new generation" sequencing (NGS) is used to search for less common genetic variants in these genes. The NGS method allows you to sequence thousands of DNA molecules simultaneously, thereby increasing the speed and efficiency of research and volume of data obtained. The philosophy of NGS is based on mass simultaneous sequencing of specially prepared single-stranded libraries of fragmented DNA obtained from samples of the material being studied. After sequencing, the data obtained are processed using bioinformatic methods. NGS technology allows you to sequence the entire genome and its encoding part—the exome—and to use panels that include only the necessary target genes, increasing productivity and simplifying the process and interpretation of the data obtained.

In this study, the following genes were sequenced: *TP53*, *MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*, *APC*, *MUTYH*, *CDKN2A*, *ATM*, *CDH1*, *PRSS1*, *CFTR*, *BRCA1*, *BRCA2*, *FANCI*, *FANCL*, *PALB2*, *RAD51B*, *RAD51C*, *RAD54L*, *RAD51D*, *CHEK2*, *CDK12*, *FANCJ/BRIP1*, *PPP2R2A*, *BARD1*, *POLE*, *POLD1*, *SMAD4*, *MLH3*, *MSH6*, *PMS1*, *NBN*, *NF1*, *RB1*, *BLM*, *FANCA*, *FANCB*, *FANCC*, *FANCD2*, *FANCE*, *FANCF*, *FANCG*, *FANCM*, *SDHB*, *SDHC*, *SDHD*, *SLX4*, *BAP1*, *BUB1*, *BUB3*, *STK11*, *AKT1*, *ATR*, *BABAM1*, *BAP1*, *PMS2*, *POLD1*, *POLE*, *RAD50*, *MEN1*, *RET*, *VHL*, and *XRCC2* [9,10].

According to several studies, the 5382insC mutation (c.5266dupC) prevails in Russia, accounting for approximately 70% of all changes in the *BRCA1* gene in BC [4,11,12] and about 60% in OC [13]. Based on the results of the analysis of literature sources, we noted the data on morphological differences in tumors with different mutations of the *CHEK2* gene [14,15]. Studies have shown that *CHEK2*-dependent tumors differ from *BRCA*-associated malignancies by their receptor status. According to some authors, patients in this group are characterized by HER2-positive non-luminal and HER2-positive luminal B subtypes of BC.

The *CHEK2* gene plays an important role in DNA repair. Hereditary mutations of this gene doubles the risk of developing BC [9,16]. If mutations in the *BRCA1/2* genes are detected for patients in many cancer centers, the characteristics of *CHEK2*-associated breast tumors are less studied because of their low penetrance. Initially, Bell et al. studied mutations in the *CHEK2* gene and noted their presence in families with inherited BC, since then, the c.470T>C mutation is considered a predictor of the development of this disease [17]. In the Russian population, mutations of the *CHEK2* gene that lead to a violation of the synthesis of a protein involved in the cell cycle are most often detected—c.444+1G>A and c.1100delC [16].

The risk of developing BC increases in the presence of mutations in the *PALB2* gene than in the *BRCA2* gene [18, 19]. Mutations in the *PALB2* gene lead to the development of a wide variety of cancer diseases. *PALB2* maintains *BRCA2* stability, and *BRCA1* regulates the movement of *PALB2* and *BRCA2* to damaged chromatin. These three proteins form a complex in which *PALB2* acts as a link between *BRCA1* and *BRCA2*.

In addition, the PALB2 protein binds a single-stranded DNA and directly interacts with RAD50 recombinase realizing homologous recombination. This complex is crucial for initiating homologous recombination in response to DNA damage.

Studies by several authors indicate a family history and high penetration of mutations in the *RAD50* gene, which encodes proteins of the MRE11-RAD50-NBN complex involved in the cellular response to DNA damage and maintenance of genome stability [20-23].

The study aimed to evaluate the spectrum and frequency of mutations in the genes of hereditary BC of patients from RB.

Materials and methods. The research is based on the analysis of the results of a molecular genetic examination of 100 unrelated patients diagnosed with BC (conducted in the State Medical Center of the Ministry of Health of RB). The study included patients with hereditary signs of cancer. Collection and analysis of the genealogy of each patient allowed us to assume the hereditary nature of BC. To do this, a patient survey was conducted. The main difficulty in obtaining the material was the lack of information of the proband's relatives. At the same time, a thorough survey of the patient's family members, among whom malignant neoplasms were registered, made it possible to distinguish the studied group of patients.

The study of a genealogy revealed from one to three blood relatives with malignant neoplasms in each family. Relatives of probands have malignant neoplasms of various localizations: breast, ovaries, uterine body, stomach, lung, colon, pancreas, prostate, and esophagus.

The age of patients ranged from 26 to 73 years, and the average was 49.9 ± 0.94 years. The patients were examined and treated for BC in the Republican Clinical Oncological Dispensary of the Ministry of Health of RB in 2016–2018, and they signed an informed consent to conduct a molecular genetic study.

To analyze the hereditary component and establish the patterns of its inheritance in families, we used the clinical and genealogical method with the compilation and analysis of genealogies. All patients included in this study had verified BC: 73 (79.0%) invasive ductal carcinoma, 25 (25.0%) invasive lobular carcinoma, and 2 (2.0%) medullary carcinoma. In the examined group of patients, cardiovascular system diseases (hypertension and coronary heart disease) were more often registered in 45 (45.0%) of the concomitant extragenital diseases, gastrointestinal tract diseases (chronic gastritis and chronic cholecystitis) in 30 (30.0%), and blood diseases (anemia) in 20 (20.0%) women. A total of 25 (25.0%) patients from gynecological pathology had a history of uterine fibroids, and 20 (20.0%) had ovarian tumors.

Results. According to immunohistochemical studies, the following molecular and biological subtypes of BC were established: 28 (28.0%) luminal A subtype, 28 (28.0%) basal-like (triple negative) subtype, 24 (24.0%) HER2-positive luminal subtype, 14 (14.0%) HER2-negative luminal B subtype, and 6 (6.0%) HER2-positive non-luminal subtype.

All patients were screened for the presence of the most common mutations in the Russian population after diagnosis and immunohistochemical studies. In tumor tissue blocks obtained after biopsy, mutations in the genes predisposing to hereditary BC were determined—*BRCA1* (185delAG, 4153delA, 5382insC, 3819delGTAAA, 3875delGTCT, 300T>G, and 2080delA) and *BRCA2* (6174delT)—by a real-time PCR method using reagent kits manufactured by DNA-Technology LLC in accordance with the recommendations of a manufacturer.

Using this method, 12 (12.0%) patients with tumor tissue were found to have mutations in the *BRCA1* gene that is included in the standard diagnostic panels. The 5382insC mutation was found in the group of detected changes. Two of these patients had primary multiple neoplasms: one had a metachronous cancer of both mammary glands, and the other had BC and OC. A patient with metachronous primary multiple BC noted the manifestations of cancer in the left and right breasts at the age of 44 and 49 years, respectively. In the second proband, the manifestation of right BC occurred at the age of 44 years and OC at the age of 61 years. None of the patients were carriers of the 6174delT mutation in the *BRCA2* gene that was included in the panel that we used.

The next stage of the study was NGS. DNA was isolated from the peripheral blood of patients, which allowed us to identify rare mutations in other genes. The NGS method confirmed mutations of patients with the *BRCA1* gene that were previously detected by PCR in tumor tissue, which confirms the hereditary nature of BC in this group.

During the study, 16 (16.0%) patients were identified with highly penetrant germinal mutations,

Table 1. Identified mutations and morphological subtype of tumor tissue of patients with BC

Gene	Morphological subtype of the tumor	Research method	Mutation	Number of patients	Percentage of mutations detected (%)
BRCA1	Basal-like	PCR (NGS)	5382insC	12	46.4
	Basal-like	NGS	c.3143delG	1	
BRCA2	Basal-like	NGS	c.6621_6622del	2	10.7
	Basal-like	NGS	c.39-1_39delGA	1	
CHEK2	HER2-positive luminal B	NGS	c.470T>C	3	17.9
	HER2-positive non-luminal	NGS	c.444+1G>A	2	
PALB2	Basal-like	NGS	c.1592delT	4	14.3
RAD50	HER2-negative luminal B	NGS	c.2157delA	2	10.7
	Luminal A	NGS	c.2157delA	1	

PCR, polymerase chain reaction; NGS, new generation sequencing.

and their ages ranged from 26 to 64 years. Heterozygous pathogenic variants in the *BRCA1* and *BRCA2* genes were detected in four (4.0%) patients (with a triple negative subtype). Although less common in the European population, c.3143delG mutation in the *BRCA1* gene was detected in one patient with a manifestation age of 57 years. The c.6621_6622del mutation in the *BRCA2* gene was identified in two patients. The age of BC manifestation was 26 and 35 years. A 45-year-old female patient with a family history (her maternal grandmother had BC, and her mother's sister had OC) had a C splicing site.-39-1_- 39delGA mutation in the *BRCA2* gene. This mutation, characterized by splicing in the intron regions of the gene, is rare and occurs mainly in the Asian population [24]. In five (5.0%) patients with different ages (from 36 to 48 years), three of whom had HER2-positive luminal B subtype and two had HER2-positive non-luminal and c.470T>C and c.444+1G>A mutations in the *CHEK2* gene, respectively. Four (4.0%) patients with a triple negative subtype of BC aged 45, 52, 60, and 64 years had the c.1592delt mutation in the *PALB2* gene. Three (3.0%) patients aged 40, 50, and 55 years had the c.2157delA mutation in the *RAD50* gene, of whom two were with HER2-negative luminal B and one luminal A subtype.

The average ages of BC manifestation in the group of patients with detected mutations and pathogenic mutations were 42.7±2.77 and 52.8 ± ± 3.82 years. The results of the study showed that in the presence of highly penetrant mutations, the age of BC manifestation is much younger than in the general population. The mutations and morphological subtype of tumor tissue identified in this study are presented in Table 1.

From the data obtained, it follows that in the group of probands, the proportion of mutations from the detected total was in the genes: *BRCA1*, 46.4%; *BRCA2*, 10.7%; *CHEK2*, 17.9%; *PALB2*, 14.3%; *RAD50*, 10.7%.

CONCLUSIONS

1. A PCR study revealed that the 5382insC (c.5266dupC) mutation in the BRCA1 gene was most frequent in 12 (12.0%) patients. In turn, the use of the NGS method has significantly improved the effectiveness of detecting mutations in BC patients. The results of the study showed that 28 (28.0%) patients had pathogenic mutations in the BRCA1, BRCA2, CHEK2, PALB2, and RAD50 genes, predisposing to the development of cancer. As a result of using the NGS method, 16 (16.0%) patients additionally revealed rare germinal mutations that determine BC development.

2. A geneticist must be consulted when identifying mutations in each patient, making up their genealogy, and determining relatives who are likely carriers of mutations. They are informed about the need to undergo a molecular genetic study to identify the corresponding mutations.

3. Medical and genetic counseling of patients with pathogenic mutations, as well as their blood relatives, makes it possible to personalize treatment and conduct prevention and early diagnosis of hereditary BC, which in turn will lead to stabilization and reduction of the incidence and mortality from this cancer pathology.

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N.I.S. and A.F.N. conducted the experimental work and made the introduction of an intellectual content; V.A.P. analyzed scientific work and introduced intellectual content; K.V.M. analyzed scientific work and developed the concept of the scientific work; SH.I.M. analyzed scientific work and made critical revision with the introduction of intellectual content; I.R.M. developed the concept of scientific work and performed bioinformatic data analysis; I.R.G. analyzed scientific work and made critical revision with the introduction of intellectual content; A.A.I. made a critical review with the introduction of an intellectual content; A.V.S. developed the concept of the scientific work with the introduction of intellectual content, clinically designed the study, and wrote an article.

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