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ONCOLOGY

Exome Sequencing: the Search for Mutations Associated with Hereditary Breast and Ovarian Cancers in the Tuvan Ethnic Group (A Pilot Study)

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Whole exome sequencing of peripheral blood samples from Tuvan females diagnosed with breast and ovarian cancers (BC/OC) was performed to search for new genes involved in BC/OC pathogenesis. Considering the high cost of whole exome sequencing and study material requirements, 9 samples were selected from 61 genomic DNA samples. A mutation in the *LGR4* gene (*rs34804482*) involved in the tumor-mediated Wnt signaling pathway and a mutation in the *BRWD1* gene (*rs147211854*) involved in chromatin remodeling were identified in BC patients. A mutation in the *CITED2* gene (*rs77963348*) involved in the pathogenesis of primary ovarian insufficiency was identified in a patient with OC and a history of infertility. A mutation in the *PDGFRA* gene (*rs2291591*) was identified in two BC/OC patients. *LRG4*, *BRWD1*, *PDGFRA*, and *CITED2* germline pathogenic mutations were discovered in Tuvan women diagnosed with BC/OC for the first time.

Key Words: *germline mutations; breast cancer; ovarian cancer; ethnic groups; Tuvans*

Malignant tumors of the female reproductive system (breast/ovarian cancers, BC/OC) are characterized by the highest incidence rate around the world [1]. An important risk factor for BC/OC is the existence of *BRCA1/2* pathogenic mutations that are inherited in an autosomal dominant manner [2]. Every population/ethnic group has a specific spectrum of mutations in its gene pool and diverse phenotypic and clinical presentations of malignancies [3]. For example, among the non-Slavic population of Russia, which involves more than 200 ethnic groups, frequent mutations in the *BRCA1/2* genes (*5382insC*, *185 delAG*, *4153 delA*, *T300G*, *3819delGTAAA*, *3875delGTCT*, *2080delA*, and *6174delT*) have been found not to be typical of BC/OC patients from the Buryat, Tuvan, Yakut, Altai, Khakas, and other ethnic groups [4]. According to published reports, *RAD51D* (*rs137886232*) and *PTEN* (*rs786201044*) pathogenic mutations in Buryat women, a *BRCA2* mutation (*rs483353122*) in Tuvan women, an *RAD54L* mutation (p.His340IlefsTer8, c.1018del, chr1:46733256) in Altai women, an *ATM* mutation (*rs780619951*) in Khakas women, and a new *PALB2* mutation (NM_024675: exon1: c.47delA: p.K16Fs) in Yakut women have been

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identified using targeted sequencing [5-7]. The spectrum of identified mutations differs from that in the Slavic population. The study was conducted in groups of BC/OC patients under 50 years and/or with a positive family history. However, pathogenic mutations were found only in a few patients in the ethnic study groups, which indicates the need to use whole exome sequencing. Whole exome sequencing provides capture of coding regions of the whole genome, which will enable identification of new genes whose pathogenic mutations can provoke BC/OC. The widespread use of whole exome sequencing in routine practice of clinical laboratories is hampered by its high cost and data arrays that require high computing power [8].

Our aim was the search for new genes associated with hereditary BC/OC in the Tuvan ethnic group using exome sequencing.

MATERIAL AND METHODS

The study included patients of the Cancer Research Institute of the Tomsk National Research Medical Center diagnosed with BC/OC (n=61) aged from 25 to 52 years (mean age 37 years) and belonging to the Tuvan ethnic group. The diagnosis was morphologically verified. All patients signed informed consent to participate in this study. The study was approved by the Ethics Committee of the Cancer Research Institute of the Tomsk National Research Medical Center (Protocol No. 10 of September 24, 2021).

Patients were tested for common *BRCA1/2* mutations. A patient with the *BRCA1 c.3615_3618del* mutation was excluded. Of the remaining patients, 23 women were selected based on a strong family history; the material was tested for pathogenic mutations in 27 genes associated with familial cancer syndrome (data not shown). Based on targeted sequencing data, high cost of tests, and material requirements, 9 DNA samples from BC/OC patients were selected.

Genomic DNA was isolated from peripheral blood leukocytes. Libraries were prepared using a BGI Optimal DNA Library Prep kit. An Agilent SureSelect Human All Exon V6 kit was used for hybridization. High-throughput sequencing was performed on a DNA nanoball sequencing platform (DNBSeq-G400). Exome sequencing data were processed using the DRAGEN Bio-IT platform v.3.9.5 (Illumina) and aligned to the hg38 reference human genome. The quality of sequencing data was controlled using the MultiQC 1.11 software.

RESULTS

According to the results of numerous studies, hereditary BC/OC is only partially caused by mutations in the most studied *BRCA1/2* genes and other genes associated with familial cancer syndrome (*ATM*, *APC*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CHEK2*, *MLH1*, *MSH2*, *MSH6*, *MUTYH*, *NBN*, *PALB2*, *PMS2*, *PMS2CL*, *PTEN*, *RAD50*, *RAD51C*, *RAD51D*, *STK11*, and *TP53*), which can be explained by ethnic specificity of gene mutations, polyethnic population composition, and an insufficient depth of the detection methods (PCR, targeted sequencing). Obviously, the introduction and application of the whole-genome sequencing will expand the list of candidate genes potentially involved in the pathogenesis of familial BC/OC. For example, in this pilot study, no *BRCA1/2* pathogenic mutations were identified in BC/OC patients from the Tuvan ethnic group. For the first time, *LGR4*, *BRWD1*, *UBXN11* pathogenic mutations and *PDGFRA*, *CITED2*, and *UBXN11* pathogenic mutations were discovered in BC and OC patients, respectively. The *LRG4*, *BRWD1*, *UBXN11*, *CITED2*, and *PDGFRA* genes are functionally involved in oncogenesis processes (cell cycle regulation, genome stability, *etc.*), but their contribution to hereditary BC/OC remains to be proven (Table 1).

***PDGFRA* (Platelet-Derived Growth Factor Receptor A) gene.** In this study, a *PDGFRA rs2291591* germline pathogenic mutation (NM_001347827:exon17:c.C2345T:p.T782M) was identified for the first time in two patients from the Tuvan ethnic group. This mutation was found in a 37-year-old BC patient with a positive family history (her father was diagnosed with gastrointestinal cancer) and in a 37-year-old patient with multiple primary malignancies (OC and others). It should be noted that this variant is described in PubMed as pathogenic, and its incidence rate varies in different populations: 0.121 (African population) and 0.025 (Asian population). The *PDGFRA* gene encodes the platelet-derived growth factor receptor alpha (PDGFRa), a representative of the family of type 3 tyrosine kinase receptors that regulate proliferation, differentiation, cell growth, and development of gastrointestinal mesenchymal tumors or gastrointestinal stromal tumors (GISTs). GISTs are a highly heterogeneous group of tumors that vary in their location, size, histological cell type, grade, risk of progression, and clinical course. It was reported that 5% of GISTs are inherited diseases and are caused by germline mutations in the *PDGFR* and *C-KIT* genes [9]. GISTs with a mutation in the KIT receptor or PDGFRa are associated with tumor response to therapy using molecular-directed (targeted) drugs (imatinib mesylate, sunitinib, *etc.*). According to generally accepted concepts, GISTs are tumors of the gastrointestinal tract, intestines, and retroperitoneal space [10]. We performed in-depth analysis of the literature and found that *PDGFRA* germline mutations were reported [11], and the *PDGFRA* gene was included in the list of cancer predisposition

TABLE 1. Pathogenic Variants of Genes Identified in BC/OC Patients from the Tuvan Ethnic Group

Patient identification code	Age, years	Family history	Pathogenic variants of genes
		BC	
T20DEO	37	—	—
T21SUA	43	Grandmother: esophageal cancer	LGR4:NM_001346432:exon17:c.A2459G:p.D820G
T22OCH	34	Sister: thyroid cancer	—
T23OA	39	—	BRWD1:NM_018963:exon40:c.A5573T:p.Q1858L
T27OS	52	—	
T1642SCHO	25	—	
T1721OCHB	37	Father: gastrointestinal cancer	PDGFRA:NM_001347827:exon17:c.C2345T:p.T782M
		OC	
T25MCHE	32	—	
T28DAA	37	Multiple primary malignancies	PDGFRA:NM_001347827:exon17:c.C2345T:p.T782M CITED2:NM_001168388:exon2:c.510_536del:p.170_179del

genes. A case of multiple GISTs in the stomach and small intestine in a woman with hereditary BC/OC syndrome with a *BRCA2* mutation was described [12]. The incidence of *PDGFRA* mutations in GISTs varies from 1% in Taiwan to 16.4% in Germany, and 9-10% in Russia [9], which is obviously associated with ethnic features. Thus, we discovered a *PDGFRA rs2291591* pathogenic variant (NM_001347827:exon17:c.C2345T:p.T782M) in BC/OC patients from the Tuvan ethnic group for the first time. The clinical significance of the *PDGFRA rs2291591* variant needs to be studied in an expanded sample of patients.

***CITED2* (Cbp/P300 Interacting Transactivator with Glu/Asp Rich Carboxy-Terminal Domain 2) gene.** In a 37-year-old patient with multiple primary malignancies (OC, endometrial cancer) and a history of infertility (two IVF attempts), apart from the *PDGFRA rs2291591* mutation, a *CITED2 rs779637348* pathogenic mutation (NM_001168388:exon2:c.510_536del:p.170_179del) was identified. According to PubMed database, the incidence rate of the *rs779637348* mutation is 0.000. The *CITED2* gene is a tumor suppressor gene that plays a crucial role in fundamental cellular processes, including proliferation, apoptosis, differentiation, migration, and autophagy [13]. A mutation in the *CITED2* gene was described in non-syndromic primary ovarian insufficiency [14]. Primary ovarian insufficiency is a clinical syndrome that includes a heterogeneous group of diseases where ovarian insufficiency is associated with *FSHR*, *GALT*, *FOXL2*, *INHA*, *EIF2B*, *BMP151*, and *AIRE* germline pathogenic mutations [15]. The percentage of primary ovarian insufficiency is 1% in the Eastern European female population, 1.1% in the general US population, 1.4% in African-American women, 0.5% in the Asian ethnic group (Chinese females), and 0.1% in Japanese women. Thus, our data indirectly indicate involvement of the *CITED2 rs779637348* gene (NM_001168388:exon2:c.510_536del:p.170_179del) in the pathogenesis of inherited primary ovarian insufficiency in Tuvan women.

***BRWD1* (Bromodomain and WD Repeat Domain Containing 1) gene.** A *BRWD1 rs147211854* germline pathogenic mutation (NM_018963:exon40:c.A5573T:p. Q1858L) was identified in a patient with BC diagnosed at 39 years of age. According to the PubMed database, the *BRWD1* mutation rate is 0.006. The *BRWD1* gene encodes bromodomain-containing proteins that are involved in different cellular functions, including transcriptional activation, SWI/SNF chromatin remodeling, mRNA splicing, and DNA replication. Six driver genes in Paget's disease (*KMT2C*, *ARID2*, *FSIP2*, *CCDC168*, *CASP8AP2*, and *BRWD1*) were identified using whole exome sequencing and MutSigCV data analysis [16]. Thus, we did not find any data on *BRWD1* germline mutations in hereditary BC/OC. The incidence rate of the *BRWD1* mutation (NM_018963:exon40:c.A5573T:p. Q1858L) should be studied in an expanded sample of BC/OC patients from the Tuvan ethnic group.

***LRG4* (Leucine Rich Repeat Containing G Protein-Coupled Receptor 4) gene.** A *LGR4 rs34804482* mutation (NM_001346432:exon17:c.A2459G:p.D820G) was identified in a Tuvan patient with BC diagnosed at the age of 43 years. According to PubMed, the *LGR4* mutation (NM_001346432:exon17:c.A2459G:p.D820G) rate is 0.0000. The *LGR4* gene is a new receptor of the family of classical leucine-rich repeat-containing G-protein-coupled receptors (LGR1 is a FSH receptor; LGR2, LH receptor; and LGR3, thyrotropin receptor). LGR4 plays an important role in endocrine

and metabolic diseases, including breast dysplasia, osteoporosis, cardiometabolic diseases, and obesity [17]. Three rare missense variants in the *LGR4* gene (NM_018490.3:c.286A>G (*rs757351670*) p.Ile96Val, NM_018490.3:c.1087G>T (*rs117543292*) p.Gly363Cys, and NM_018490.3:c.2531A>G (*rs34804482*) p.Asp-844Gly) were identified in six unrelated families (17 patients) affected by delayed puberty with an autosomal dominant pattern of inheritance [18]. All six probands were males; however, in two families, mutations were identified in females. All probands had delayed onset of Tanner stage G2 with low serum gonadotropin and testosterone levels. Thus, the *LGR4* pathogenic mutation (NM_001346432:exon17:c.A2459G:p.D820G), which can be associated with breast pathology and delayed puberty, was discovered in the Tuvan women with BC for the first time.

We also identified *UBXN11* (*rs2073002071*, NM_001077262:exon11:c.1104_1181del:p.368_394del), *CDCA8* (*rs145033890*, NC_000001.10:g.38173986_38173988del), and *NQO1* (*rs1800566*, NM_001286137:exon4:c.C343T:p.P115S) pathogenic mutations. The *UBXN11* (UBX Domain Protein 11) pathogenic mutation (*rs2073002071*, NM_001077262:exon11:c.1104_1181del:p.368_394del) may be involved in the pathogenesis of malignancies, but information on this mutation is too scant to assess its significance. *CDCA8* (Cell Division Cycle Associated 8) (*rs145033890*, NC_000001.10:g.38173986_38173988del) and *NQO1* (NAD(P)H dehydrogenase 1) (*rs1800566*, NM_001286137:exon4:c.C343T:p.P115S) pathogenic mutations have a high rate in the Asian population: 0.70 and 0.44, respectively, which is significantly higher than the expected rate for a pathogenic mutation.

Therefore, we identified *PDGFRA*, *CITED2*, *LGR4*, and *BRWD1* germline pathogenic mutations in BC/OC patients from the Tuvan ethnic group. The significance of these mutations needs to be confirmed in an expanded sample of BC/OC patients (from the Tuvan ethnic group). If *PDGFRA*, *CITED2*, *LGR4*, and *BRWD1* mutations are involved in the pathogenesis of hereditary BC/OC and have clinical significance, this should be considered upon examination of patients from the Tuvan ethnic group, namely, to improve therapeutic approaches and develop prevention programs and guidelines for medical and genetic counseling (family planning). It is also necessary to develop algorithms for testing recurrent and rare variants of mutations in the Tuvan ethnic group with BC/OC, including expanded custom gene panels for high-throughput sequencing and rapid PCR-based kits.

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Conflict of interest. The authors have no conflicts of interest to declare.

REFERENCES

1. Adamyan LV, Olina AA, Grigorian IE, Asaturova AV. Oncoprophylaxis in gynecology considering the analysis of international experience. problems and solutions. Problemy Reprodukts. 2021;27(6):70-80. Russian. doi: 10.17116/repro20212706170
2. Sultanbaev AV, Nasretdinov AF, Galeev MG, Menshikov KV, MUSIN ShI, Sultanbaeva NI, Ayupov RT, Rakhimov RR, Lipatov ON, Izmaylov AA, Menshikova IA, Serebrennikov GA, Lipatov DO. Breast cancer screening in germline mutation carriers. A literature review. Opukholi. Zhensk. Reprod. Sist. 2023;19(2):16-24. Russian. doi: 10.17650/1994-4098-2023-19-2-16-24
3. Zinchenko RA, Ginter EK, Kutsev SI. Features of the diversity of hereditary diseases in different regions and multiethnic populations of the Russian Federation. Med. Genetika. 2020;19(7):13-14. Russian. doi: 10.25557/2073-7998.2020.07.13-14
4. Cherdyntseva NV, Pisareva LF, Ivanova AA, Panferova YeV, Malinovskaya EA, Odintsova IN, Doroshenko AV, Gervas PA, Slonimskaya EM, Shivitool AA, Dvornichenko VV, Choinzonov YeL. Ethnic aspects of hereditary breast cancer in the region of Siberia. Vestn. Ross. Akad. Med. Nauk. 2014;69(11-12):72-79. Russian. doi: 10.15690/vramn.v69i11-12.1186
5. Gervas P, Klyuch B, Denisov E, Kiselev A, Molokov A, Pisareva L, Malinovskaya E, Choynzonov E, Cherdyntseva N. New germline BRCA2 gene variant in the Tuvinian Mongol breast cancer patients. Mol. Biol. Rep. 2019;46(5):5537-5541. doi: 10.1007/s11033-019-04928-y
6. Gervas P, Molokov A, Schegoleva A, Kiselev A, Babyshkina N, Pisareva L, Tyukalov Y, Choynzonov E, Cherdyntseva N. New germline mutations in non-BRCA genes among breast cancer women of Mongoloid origin. Mol. Biol. Rep. 2020;47(7):5315-5321. doi: 10.1007/s11033-020-05612-2
7. Gervas PA, Molokov AYu, Zarubin AA, Ivanova AA, Tikhonov DG, Kipriyanova NS, Egorov AN, Zhuikova LD, Shefer NA, Topolnitskiy EB, Belyavskaya VA, Pisareva LF, Choynzonov EL, Cherdyntseva NV. A novel germline mutation of the PALB2 gene in a young Yakut breast cancer woman. Sib. J. Oncol. 2022;21(4):72-79. doi: 10.21294/1814-4861-2022-21-4-72-79
8. Suspitsin EN, Tyurin VI, Imyanitov EN, Sokolenko AP. Whole exome sequencing: principles and diagnostic capabilities. Pediatr. 2016;7(4):142-146. Russian. doi: 10.17816/PED74142-146
9. Mazurenko NN, Tsyganova IV. Molecular features and genetic markers of gastrointestinal stromal tumors. Uspekhi Mol. Onkol. 2015;2(2):29-40. Russian. doi: 10.17650/2313-805X.2015.2.2.29-40
10. Sidakova ZA, Baranova AA, Gritsai AN, Efremov GD, Lyubchenko LN. A clinical case of extragastrointestinal tumor in combination with testicular feminization syndrome (Morris syndrome). Sib. Onkol. Zh. 2023;22(4):142-148. Russian. doi: 10.21294/1814-4861-2023-22-4-142-148
11. Bodian DL, McCutcheon JN, Kothiyal P, Huddleston KC, Iyer RK, Vockley JG, Niederhuber JE. Germline variation in cancer-susceptibility genes in a healthy, ancestrally diverse cohort: implications for individual genome se-

quencing. PLoS One. 2014;9(4):e94554. doi: 10.1371/journal.pone.0094554
12. Sekido Y, Ohigashi S, Takahashi T, Hayashi N, Suzuki K, Hirota S. Familial gastrointestinal stromal tumor with germline KIT mutations accompanying hereditary breast and ovarian cancer syndrome. Anticancer Res. 2017;37(3):1425-1431. doi: 10.21873/anticanres.11466
13. Yu YP, Michalopoulos A, Ding Y, Tseng G, Luo JH. High fidelity copy number analysis of formalin-fixed and paraffin-embedded tissues using Affymetrix Cytoscan HD chip. PLoS One. 2014;9(4):e92820. doi: 10.1371/journal.pone.0092820
14. Venturella R, De Vivo V, Carlea A, D'Alessandro P, Saccone G, Arduino B, Improda FP, Lico D, Rania E, De Marco C, Viglietto G, Zullo F. The genetics of non-syndromic primary ovarian insufficiency: a systematic review. Int. J. Fertil. Steril. 2019;13(3):161-168. doi: 10.22074/ijfs.2019.5599
15. Chebotnikova TV. Primary ovarian failure: experts opinion. Vestn. Reprod. Zdor. 2007;(1):22-32. Russian. doi: 10.14341/brh2007122-32
16. Zhang G, Zhou S, Zhong W, Hong L, Wang Y, Lu S, Pan J, Huang Y, Su M, Crawford R, Zhou Y, Mai R. Whole-exome sequencing reveals frequent mutations in chromatin remodeling genes in mammary and extramammary Paget's diseases. J. Invest. Dermatol. 2019;139(4):789-795. doi: 10.1016/j.jid.2018.08.030
17. Zhang N, Yuan M, Wang J. LGR4: a new receptor member in endocrine and metabolic diseases. Endocr. Rev. 2023;44(4):647-667. doi: 10.1210/endrev/bnad003
18. Mancini A, Howard SR, Marelli F, Cabrera CP, Barnes MR, Sternberg MJ, Leprovots M, Hadjidemetriou I, Monti E, David A, Wehkalampi K, Oleari R, Lettieri A, Vezzoli V, Vassart G, Cariboni A, Bonomi M, Garcia MI, Guasti L, Dunkel L. LGR4 deficiency results in delayed puberty through impaired Wnt/β-catenin signaling. JCI Insight. 2020;5(11):e133434. doi: 10.1172/jci.insight.133434