Molecular Biology Reports (2019) 46:5537–5541
https://doi.org/10.1007/s11033-019-04928-y

SHORT COMMUNICATION

New germline *BRCA2* gene variant in the Tuvinian Mongol breast cancer patients

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Received: 2 January 2019 / Accepted: 18 June 2019 / Published online: 4 July 2019

Abstract

To date, there are a limited number of reports on inherited gene mutations associated with breast cancer (BC) among Mongoloid indigenous people in Russia. The present study aimed at identifying the BC-associated genes in 26 Russian Mongoloid BC patients (Buryats, Tuvinians and others). The median age of the patients at the time of breast cancer diagnosis was 41 years (range 25–51 years). Genomic DNA isolated from blood samples was used to prepare libraries using a capture-based target enrichment kit (Hereditary Cancer Solution™, SOPHiA GENETICS, Switzerland) covering 27 genes (*ATM*, *APC*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CHEK2*, *EPCAM*, *FAM175A*, *MLH1*, *MRE11A*, *MSH2*, *MSH6*, *MUTYH*, *NBN*, *PALB2*, *PIK3CA*, *PMS2*, *PMS2CL*, *PTEN*, *RAD50*, *RAD51C*, *RAD51D*, *STK11*, *TP53* and *XRCC2*). Next-generation sequencing (NGS) was performed on an Illumina NextSeq 500 System (Illumina, USA). In our study, we found 1 Indel and 11 SNPs that passed filters during variant calling. We identified a highly pathogenic germline rs483353122 (c.8208_8209insAG, p.Leu2737Serfs*2) in the *BRCA2* gene in six unrelated Tuvinian Mongol BC patients. We also identified a likely damaging germline rs35352891 in the *MUTYH* gene (c.1118C>T, p.Ala373Val) in one Buryat Mongol BC patient. Other SNPs were classified as variants of uncertain significance. To the best of our knowledge, this report is the first to describe the highly pathogenic variant in the *BRCA2* gene (rs483353122) and the likely damaging germline variant in the MUTYH gene (rs35352891) in Russian Mongoloid BC patients with young-onset and/or bilateral and/or familial BC. Further studies are therefore necessary to evaluate the contributions of novel sequence variants to hereditary BC.

Keywords BRCA1/2 · Germline mutation · Breast cancer · Mongoloid race

Introduction

Breast cancer (BC) is the most prevalent female malignancy worldwide. Mutations in the *BRCA1* and *BRCA2* genes are inherited in an autosomal dominant manner and are responsible for 5–8% of all BC cases and 20–25% of familial BC cases [1, 2]. *BRCA1* and *BRCA2* lesions have been detected in various races, ethnicities, and geographic regions, but the incidence of *BRCA1/2* mutations in high-risk families varies widely among different populations. For example, the *BRCA1* 5382insC (rs80357906) mutation has been reported in individuals of Jewish, Dutch, Lithuanian, Hungarian, Germanic, French, Italian, British, and French–Canadian ancestries [3, 4]. In Russians, who are descended primarily from Slavic ancestors (newcomers), a strong founder effect was similarly observed for the *BRCA1* 5382insC allele, which accounted for up to 90% of all known BC-associated mutations in this population [5].

Representatives of more than 200 nationalities (ethnic groups) live in Russia. Approximately 81% of the population of Russia are Russians, up to 10% of the population are Tatars, Belarusians, Ukrainians, Bashkirs, Chuvash, Chechens, Armenians and 0.5% each fall on the share of Kazakhs, Yakuts, Buryats, Ingush, Udmurts, Ossetians and

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others. The incidence of BC in all age groups of the indigenous population is lower than that of the newcomers. In the indigenous population, the incidence peak was observed at age 50–59. In newcomers, the incidence reached the peak at 60–69 years [6]. To date, there are a limited number of reports on the inherited gene mutations associated with BC among Mongoloid indigenous peoples in Russia. However, racial/ethnic minority individuals have a significant burden of cancer and limited access to genetic cancer risk assessments. Nevertheless, *BRCA* testing is a standard of care for breast and/or ovarian cancer patients with a family history [7]. The present study aimed at identifying mutations of BC-associated genes in 26 Mongoloid BC patients (Tuvinians, Buryats and others).

Materials and methods

Inclusion criteria included the Mongoloid ethnicity BC patients aged 25–51 years with or without family history. Exclusion criteria included the presence of well-known BC-associated *BRCA* gene mutations (*BRCA1* 5382insC, 185delAG, 4153delAG, T300G, 3819delGTAAA, 3875delGTCT, 2080delA and *BRCA2* 6174delT).

Twenty-six patients were included in the study. The median age of the patients at the time of BC diagnosis was 41 years (range, 25–51), 42% of patients were 39 years old or younger (Table 1). The study was conducted in accordance with the Helsinki Declaration (1964). The Ethics Committee of the Cancer Research Institute (Tomsk NRMC) approved the study, and all participants provided written informed consent.

Table 1 The Mongol BC patients enrolled into the study

Sample	Age at diagnosis	Disease	Family history	Ethnicity	NGS data
1	39	BC only	Aunt—BC, CRC	Tuvan	–
2	34	BC only	Mother—BC	Tuvan	–
3	49	BC only	Aunt—BC	Tuvan	*BRCA2* (rs483353122)
4	51	BC only	Mother—BC	Tuvan	*BRCA2* (rs483353122)
5	45	BC only	Family has no history of cancer	Tuvan	*MUTYH* (rs199840380) *MRE11A* (rs372131911)
6	43	BC only	Family has no history of cancer	Tuvan	
7	33	BC only	Family has no history of cancer	Tuvan	
8	41	BC only	Family has no history of cancer	Tuvan	*BRCA2* (rs483353122) *MUTYH* (rs199840380)
9	25	BC only	Family has no history of cancer	Tuvan	
10	39	BC only	Unknown	Tuvan	*APC* (rs762117133)
11	39	BC only	Unknown	Tuvan	*MUTYH* (rs199840) *BRCA2* (rs80359254)
12	37	BC only	Father—colorectal cancer	Tuvan	*MLH1* (rs104894994)
13	44	BC only	Family has no history of cancer	Tuvan	–
14	42	Bilateral BC only	Grandmother, aunt—stomach cancer	Tuvan	–
15	41	BC only	Unknown	Tuvan	–
16	43	Bilateral BC only	Unknown	Tuvan	–
17	39	BC only	Unknown	Tuvan	*BRCA2* (rs483353122)
18	40	BC only	Unknown	Tuvan	
19	45	BC only	Unknown	Tuvan	*BRCA2* (rs483353122)
20	38	BC only	Unknown	Tuvan	*BRCA2* (rs483353122)
21	50	BC only	Grandmother—OC, aunt—BC, mother—RCC	Buryat	*MUTYH* (rs35352891)
22	35	BC only	Sister—OC, father—stomach cancer	Buryat	*MLH1* (rs4986984)
23	37	BC only	Mother—RCC, sister—BC	Buryat	*NBN* (rs746381477)
24	45	BC only	Sister—BC, brother—CRC	Khakas	*ATM* (rs587781365) *APC* (rs748715887)
25	47	BC only	Mother—BC	Yakut	–
26	42	BC only	Aunt—BC	Altaian	–

BC breast cancer, *CRC* colorectal cancer, *RCC* renal cell carcinoma, *OC* ovarian cancer

Blood samples were collected in EDTA-containing tubes, genomic DNA was extracted according to the user manual (phenol/chloroform method). DNA purity was measured using UV spectroscopy (NanoDrop 1000 spectrophotometer, ThermoFisher Scientific, USA), where the A260/A280 and A260/A230 ratios are indicators of different contaminants. DNA concentrations were measured with a Qubit® dsDNA HS Assay Kit (ThermoFisher Scientific, USA). DNA integrity (DIN) was verified on an 2200 TapeStation system (Agilent, USA).

Library preparation was performed using a capture-based target enrichment kit Hereditary Cancer Solution™ (SOPHiA GENETICS, Switzerland) covering 27 genes (*ATM*, *APC*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CHEK2*, *EPCAM*, *FAM175A*, *MLH1*, *MRE11A*, *MSH2*, *MSH6*, *MUTYH*, *NBN*, *PALB2*, *PIK3CA*, *PMS2*, *PMS2CL*, *PTEN*, *RAD50*, *RAD51C*, *RAD51D*, *STK11*, *TP53* and *XRCC2*). NGS sequencing (150 bp paired-end) was performed on a NextSeq 500 System (Illumina, USA). The variants identified by the NGS approach were confirmed by Sanger sequencing analysis and variant calling were performed using the SOPHiA DDM® platform (SOPHiA GENETICS, Switzerland). The average coverage for all 27 genes ranged from 1300 to 1700×. Allele frequency values were taken from the 1000 Genomes project (1000G; Based on Project Phase III Data), Exome Variant Server (NHLBI Exome Sequencing Project), Exome Aggregation Consortium (ExAC), and the SOPHiA DDM® Platform.

Sequencing data was also analyzed according to GATK best practice recommendation for WES, using GRCh37 as reference for BWA-mem alignment. Obtained variants were annotated with Annovar software, and ranged according to population frequency (GnomAD exome, GnomAD genome, ExAC), LoF, Clinvar, CADD and literature data [8–10]. The clinical significance of amino acid substitution was assessed using Polyphen 2, Mutation Taster databases, and SIFT programs.

Results and discussion

The currently available data support the hypothesis that each geographic region has its own *BRCA* alterations. The 5382insC mutation in the *BRCA1* gene is the most common alteration in Eastern and Central Europe, including Russia (the Central and Siberian regions). *BRCA* gene mutations that are widespread in many regions (e.g., 5382insC and 185delAG) cannot be used as universal markers of BC predisposition in all geographic regions. In Siberia and the Russian Far East, the frequency of the 5382insC mutation is 3.5% among newcomers with Slavic ancestry; however, no mutations in the *BRCA* genes (*BRCA1* 5382insC, 185delAG, 4153delAG, and T300G, and *BRCA2* 6174delT mutation) have been identified among Mongoloid indigenous people in Russia [11].

In our study, we screened 26 unrelated BC patients of Mongoloid decent with young-onset and/or bilateral and/or familial BC. The identified genetic variants are shown in Table 2. The table provides a functional annotation of 1 Indel and 11 SNPs that pass filters during variant calling. All polymorphisms were found in a heterozygous state.

The frameshift rs483353122 variant (c.8208_8209insAG, p.Leu2737Serfs*2) in the *BRCA2* gene in the heterozygous state was identified in six unrelated Tuvinian Mongol BC patients (Fig. 1). Table 1 demonstrates data on the family history of patients with the *BRCA2* rs483353122 pathogenic variant. The rs483353122 variant in the *BRCA2* gene was previously mentioned in the dbSNP and was classified as highly pathogenic. This variant was not present in the 1000 Genomes Project (1000G; Based on Project Phase III Data), Exome Aggregation Consortium (ExAC) and SOPHiA DDM® platform. This variant was initially identified in the Research Molecular Genetics Laboratory, Women's College Hospital, and University of Toronto and was characterized as germline. The variant was also identified as germline in the Chinese population of the Hksar geographic origin. The rs483353122 variant was described in another study for the Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA, University of Cambridge). This variant was characterized as germline by Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA).

We also identified a likely damaging germline variant in the *MUTYH* gene (rs35352891, c.1118C>T, p.Ala373Val) in one Buryat Mongol BC patient aged 50. According to the ClinVar database, the clinical significance of the allelic variant is controversial. However, according to PolyPhen-2, the clinical significance of the allelic variant is probably pathogenic. In our study, a 50-year-old Buryat patient with a hereditary history (mother—colorectal cancer, grandmother—ovarian cancer, aunt—BC) was diagnosed with invasive ductal breast carcinoma (T2N0M0). Considering that the patient with burdened family history was diagnosed with BC at the age of 50 years, rs35352891 of the *MUTYH* gene may be a BC-associated variant with a low effect of penetration. This report is the first to describe rs35352891 in the *MUTYH* gene in Buryat BC patient with a family history of disease.

Other SNPs were classified as variants of uncertain/unknown significance or likely benign.

It should be noted that this study has two primary limitations. First, we were not able to include families with two or more female relatives with hereditary BC. Second, our findings require authentication in representative groups of patients.

Table 2 Functional annotation of the identified genetic variants in the Mongol BC patients

Gene/nucleotide change (HGVS)	Amino acid change	Mutation type	SNP	Pathogenicity dbPubMed
APC c.4928G>A	p.Cys1643Tyr	M	rs748715887	US
APC c.775C>A	p.Arg259Trp	M	rs762117133	US
ATM c.7592T>C	p.Met2531Thr	M	rs587781365	US
BRCA2 c.8208_8209insAG	p.Leu2737Serfs*2	F	rs483353122	HP
BRCA2 c.9934A>G	p.Ile3312Val	M	rs80359254	US
MLH1 c.649C>T	p.Arg217Cys	M	rs4986984	US
MLH1 c.-523C>T	–	UTR-5	rs104894994	US
MUTYH c.1118C>T	p.Ala373Val	M	rs35352891	Conflicting/likely damaging
MUTYH c.838C>T	p.Arg281Cys	M	rs199840380	US
MUTYH g.22380294T>G	–	–	rs199840	US
MRE11A c.358A>G	p.Ile120Val	M	rs372131911	US
NBN c.880A>T	p.Met294Leu	M	rs746381477	US

HGVS Mutation type according to the Human Genome Variant Society nomenclature, *M* missense, *UTR-5* five prime untranslated region, *F* frameshift mutation, *US* unknown significance, *HP* highly pathogenic

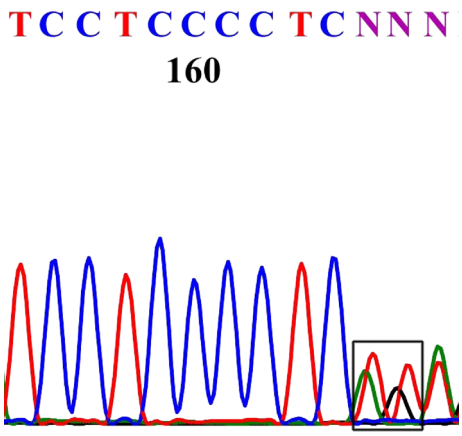

Fig. 1 Sequenogram of exon 18 of the *BRCA2* gene (rs483353122, c.8208_8209insAG, highly pathogenic variant). The sequenogram illustrates the insertion of AG nucleotides

Conclusion

To the best of our knowledge, this report is the first to describe the highly pathogenic variant in the *BRCA2* gene (rs483353122) and the likely damaging germline variant in the MUTYH gene (rs35352891) in Russian Mongoloid BC patients with young-onset and/or bilateral and/or familial BC. Further studies are therefore necessary to evaluate the contributions of novel sequence variants to hereditary BC.

Acknowledgements We acknowledge the Tomsk State University for the Competitiveness Improvement Program. The work with DNA sequencing was performed on equipment from the Tomsk regional common use center with the support of the Russian Ministry; Agreement No. 14.594.21.0001 (RFMEFI59414X0001). This study was funded by Russian Foundation for Basic Research, 18-29-09046.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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