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ORIGINAL ARTICLE

New germline mutations in non-*BRCA* genes among breast cancer women of Mongoloid origin

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Abstract

In accordance with the Asian *BRCA* Consortium data, there is a significant difference in incidence rate of breast cancer depending on age, as well as spectrum and prevalence of *BRCA1/2* mutations between Mongoloid (East Asian) and Caucasoid (European) people. However, European strategies to identify familial BC are still applied to the Asian population, including Russian Mongoloids (Khakas, Buryats, Tyvans and Yakuts and others). The main purpose of the study was to identify molecular changes associated with hereditary BC in Russian Mongoloid BC patients (Buryats). Thirty-nine patients were included in the study. Genomic DNA extracted from lymphocytes was used to prepare DNA-libraries. Target sequencing was designed to cover 27 genes, such as *ATM, APC, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2* and others. Paired-end sequencing (2 × 150 bp) was conducted on a NextSeq 500 system (Illumina, USA). Three pathogenic mutations in non-*BRCA* genes were found (prevalence of 8%). The pathogenic mutations were found in the *RAD51D* and *PTEN* genes. The pathogenic variant in the *RAD51D* gene (rs137886232, NC_000017.10:g.33428366G>A, p.R141X) was observed in two unrelated individuals aged under 40. One of these patients had a family history of late-onset stomach cancer in second-degree relatives. The pathogenic mutation in the *PTEN* gene (rs786201044, NC_000010.10:g.89692922T>C, p.C136R) was observed in a 38 years old breast cancer patient with no family history. In our study, we first describe pathogenic mutations in *RAD51D* and *PTEN* genes found in young Buryat patients.

Keywords Germline mutation · Breast cancer · Mongoloid race

Introduction

Breast cancer (BC) is the most common female malignancy worldwide. Mutations in *BRCA1* and *BRCA2* genes are responsible for hereditary BC. Individuals with inherited mutations in *BRCA* genes should be offered the risk-reduction strategies, such as screening (mammography and breast magnetic resonance imaging), surveillance (clinical breast examination, breast self-examination), chemoprevention, and risk-reduction surgery [1].

BC is also caused by mutations in the *TP53, ATM, CDH1, PTEN*, and *STK11* genes associated with hereditary syndromes. Mutations in the genes mentioned above can inhibit DNA repair pathways [2, 3].

In Russians, mutations of *BRCA1/2* genes were found only among Slavic women (newcomers), and were not found in Khakas, Buryats, Tyvans, Yakuts and others women (indigenous) [4, 5]. However, the cancer burden in Khakas, Buryats, Tyvans has risen and the cancer risk assessment has been limited [6–8]. The main purpose of the study was to identify molecular changes associated with hereditary BC in Russian Mongoloid BC patients.

Materials and methods

Thirty-nine patients were included in the study. The median age of patients at BC diagnosis was 42 years (range: 26–55). Eighty-one percent of patients were diagnosed with BC

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before the age of 50. More than one-third of patients under the age of 50 had a family history of BC. Almost all tested women were diagnosed with invasive (ductal) carcinoma of no special type. Information, including age at diagnosis, family history, histological type of cancer, and family origin was obtained. Reported clinical characteristics of the patients are given in Table 1.

Blood samples were collected in ethylenediaminetetraacetic acid-containing tubes. Genomic DNA from peripheral blood was extracted using the phenol–chloroform method. Purity of the DNA was assessed using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, USA) and then quantified using the Qubit 2.0 fluorometer and HS dsDNA Assay Kit (Thermo Fisher Scientific, USA). Integrity of the DNA (DIN) was verified on a 2200 TapeStation system (Agilent, USA). The positive control sample with *BRCA1* c.3755_3758delTGTC pathogenic mutation was included as an inner control.

DNA library were prepared using the Hereditary Cancer Solution™ kit (Sophia GENETICS, Switzerland) to cover 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*. Paired-end sequencing (2 × 150 bp) was conducted using NextSeq 500 system (Illumina, USA). The pathogenic variants were validated using Sanger sequencing (SeqStudio, Thermo Fisher Scientific, USA).

Bioinformatics analysis

Sequencing data was analyzed according to the GATK best practice recommendation for Whole Exome Sequencing using GRCh37 as a reference for Burrows-Wheeler alignment. The obtained variants were annotated with ANNOVAR software and ranged according to population frequency (genomic exome, gnomAD genome, and ExAC), ClinVar, CADD, and literature data [9–11]. Detected sequence variants were annotated using PolyPhen2, Mutation Taster, and SIFT [12–14].

Table 1 Clinical characteristics of the study cohort

Characteristics	n (%)
Age of diagnosis (years)	
< 45	18 (46)
45–49	14 (35)
50–55	7 (19)
Histologic type	
Invasive (ductal) carcinoma of no special type	37 (95)
Invasive lobular carcinoma	2 (5)
Tumor side	
Left	12 (31)
Right	21 (53)
Unknown	6 (16)
Lymph nodes status	
Positive	17 (43)
Negative	10 (26)
Unknown	12 (31)

Results

In our study, 8% (3/39) of patients harbored one pathogenic variant and 15% (6/39) of patients harbored likely pathogenic variant. In addition, 8% of patients had VUS, 15% had conflicting variants and 54% had only benign variants (Fig. 1a).

It should be noted that the pathogenic variants were found in two non-*BRCA1/2* susceptibility genes and were diagnosed only in BC patients under 45 years old (Figs. 1b, 2). Table 2 illustrates variants that are described as highly pathogenic by dbPubMed, likely pathogenic (possibly/probably damaging by PolyPhen2 or deleterious by SIFT).

The *RAD51D* variant (rs137886232) was observed in two unrelated individuals. One of these patients had a family history of late-onset stomach cancer in second-degree relatives. Another pathogenic variant was observed in the *PTEN* gene (rs786201044) in a BC patient aged 38 with no family history of BC.

Variants in the *ATM*, *MSH6* and *MLH1* genes (likely pathogenic) were previously described as VUS by dbPubMed, but were predicted as probably damaging by PolyPhen2 and/or deleterious by SIFT. The probably damaging variants (PolyPhen2) in the *ATM* (rs150757822) and *MSH6* (rs142254875) genes were observed in a 49-year-old patient with a burdened family history and in a 48-year-old patient with an unknown family history, respectively. One *MLH1* variant (rs4986984) was classified as probably damaging (PolyPhen2) and deleterious (SIFT), and was found in two patients diagnosed with BC prior to 46 years and in a 52-year-old patient with a burdened family history. Another probably damaging/deleterious variant in the *MLH1* gene (rs367654552) was found in a 55-year-old patient with a burdened family history.

Rare genetic variants classified by dbPubMed as VUS are given in Table 3. All variants presented in Table 3 were considered benign by PolyPhen2 or tolerated by SIFT. One missense VUS (rs80359254) was identified in the *BRCA2* gene. VUS were most commonly encountered in the *ATM* (n = 3), *MSH6* (n = 2) and *MLH1* (n = 3) genes.

The rs367654552 of the *MLH1* gene was previously described only in the East Asia, whereas the rs80359254 variant of the *BRCA2* gene was observed exclusively in Europeans. The rs150757822 and rs1800058 variants of

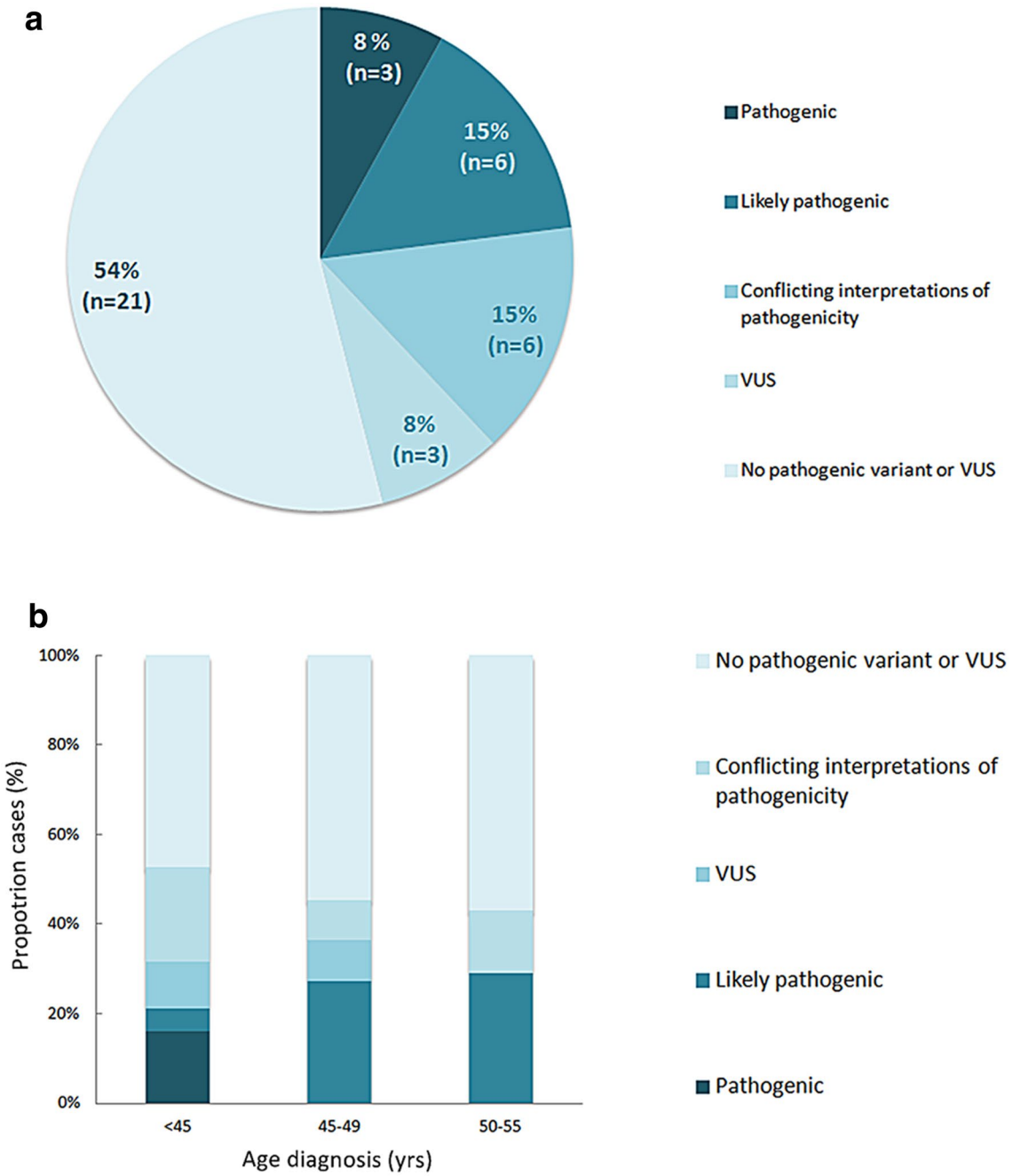

Fig. 1 **a** Proportion of unrelated BC patients by functional status of the observed gene variants in the coding regions and **b** distribution after grouping by age of onset

the *ATM* gene and the rs104894994 variant of the *MLH1* gene were found in South Asian populations but not in the East Asian populations. In general, 77% (10 out of 13) of the identified variants were previously found among Europeans (Table 4).

No mutations were found in the *MRE11A, PIK3CA, RAD51C* or *XRCC2* gene.

4. Discussion

Mongoloid population is the most prevalent among all human populations [15]. In accordance with the Asian *BRCA* Consortium data, there is a significant difference in incidence rate of BC depending on age, as well

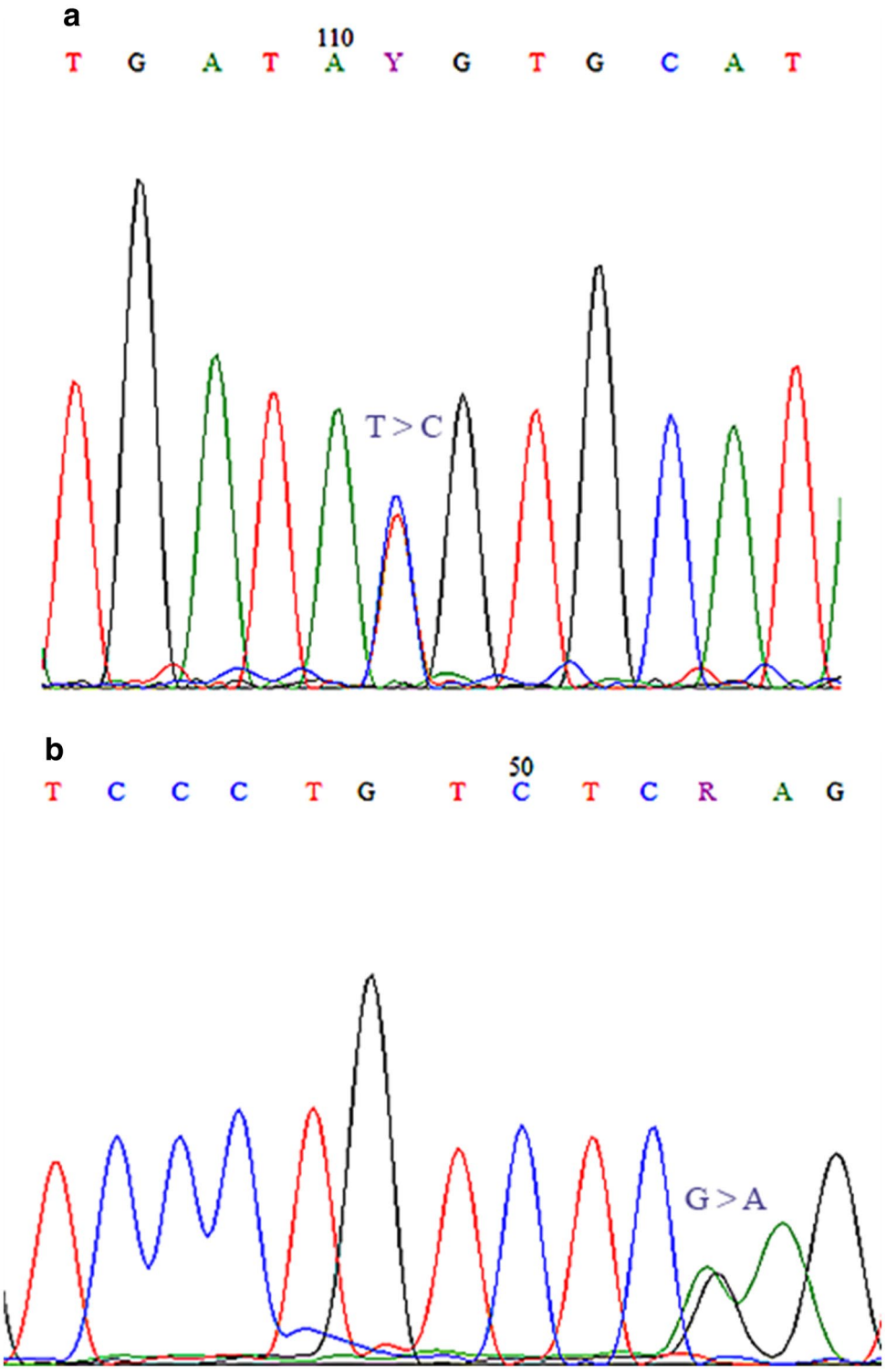

Fig. 2 Sequenograms of the RAD51D (rs137886232, NC_000017.10:g.33428366G>A, pathogenic variant) (**a**) and PTEN (rs786201044, NC_000010.10:g.89692922T>C, pathogenic variant) (**b**) genes

as spectrum and prevalence of *BRCA1/2* mutations and clinical significance of rare VUS between Mongoloid (East Asian) and Caucasoid (European) people [16–20]. However, European strategies to identify familial BC are still applied to the Asian population, including Mongoloids.

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

Gene	db SNP ID	HGVS	Amino acid change (HGVS)	dbPubMed	PolyPhen2	SIFT
ATM	rs150757822	NC_000011.9: g.108183194A>C	p.Lys1992Thr	VUS	Possibly damage	Tolerated
MSH6	rs142254875	NC_000002.11: g.48030603C>G	p.Pro1073Ala	VUS	Possibly damage	Tolerated
MLH1	rs4986984	NC_000003.11: g.37053562C>T	p.Arg217Cys	VUS	Probably damaging	Deleterious
	rs367654552	NC_000003.11: g.37035090C>G	p.Arg18Gly	VUS	Probably damaging	Deleterious
PTEN	rs786201044	NC_000010.10: g.89692922T>C	p.Cys136Arg	HP	–	–
RAD51D	rs137886232	NC_000017.10: g.33428366G>A	p.Arg253Ter	HP	–	–

SNP single nucleotide polymorphism, *HGVS* mutation type according to the Human Genome Variant Society nomenclature, *VUS* variants of unknown significance, *HP* highly pathogenic, *PolyPhen2* polymorphism phenotyping version 2; probably damaging, i.e., it is with high confidence supposed to affect protein function or structure; possibly damaging, i.e., it is supposed to affect protein function or structure; *SIFT* sort intolerant from tolerant

Table 3 Variants of uncertain clinical significance (VUS)

Gene	db SNP ID	HGVS names	Amino acid change (HGVS)	MAF	Mutation type	Pathogenicity
ATM	rs139379666	NC_000011.9: g.108235879C>T	p.Pro2974Leu	T = 0.00010/12 (ExAC)	M	Conflicting
	rs1800058	NC_000011.9: g.108160350C>T	p.Leu1420Phe	T = 0.0127/1457 (ExAC)	M	VUS
BRCA2	rs80359254	NC_000013.10: g.32972584A>G	p.Ile3312Val	G = 0.000008/1 (ExAC)	M	VUS
MSH6	rs61756469	NC_000002.11: g.48010479C>T	p.Ala36Val	T = 0.0002/17 (ExAC)	M	VUS
MLH1	rs104894994	NC_000003.11: g.37035032C>T	–	T = 0.0015/180 (ExAC)	UTR5	Conflicting

SNP single nucleotide polymorphism, *HGVS* mutation type according to the Human Genome Variant Society nomenclature, *MAF* minor allele frequency, *ExAC* Exome Aggregation Consortium, *M* missense, *UTR-5* five prime untranslated region, *VUS* variants of unknown significance, *US* unknown significance

There are more than 200 different ethnic groups in Russia. Most of the population in Russia includes Russians (81%), the largest ethnicities are Tatars, Belarusians, Ukrainians, Bashkirs, Chuvash, Chechens, and Armenians (up to 10%), and smallest nationalities include Kazakhs, Yakuts, Buryats, Ingush, Udmurts, Ossetians and others (up to 0.5% of each) [5]. In our study, we continued to search for mutations in *BRCA*-negative BC women living in Russia (Buryat). Overall, two pathogenic germline variants in *RAD51D* and *PTEN* were found in 8% (3/39) of patients under 40. In addition, 8% of patients had VUS, 15% had conflicting variants and 54% had only benign variants.

The pathogenic variant of *RAD51D* gene (rs137886232) with low minor allele frequency was observed in two young unrelated Buryat patients. The germline mutation of *RAD51D* gene (rs137886232) was suggested to have a founder effect in Chinese population [21]. This variant was also described in BC families of European ancestry. It was also reported that *RAD51D*-deficient tumor cells were sensitive to poly-(ADP) ribose polymerase inhibitors [22].

The pathogenic variant of the *PTEN* gene (rs786201044), which was predicted to be damaging by in silico analysis, was observed in BC patient aged 38 with no family history of BC. Different studies also reported on this variant (rs786201044) of the *PTEN* gene in families with Cowden syndrome (an autosomal dominant inherited disorder) [23–26]. Previous studies have also found that this variant (rs786201044) of the *PTEN* gene may impact on protein stability and lead to increased proteasome activity [27–31].

Interesting, germline variants of *RAD51D* and *PTEN* genes were also described in the COSMIC database as

Table 4 Frequencies of non-synonymous pathogenic variants and VUS in different populations

Gene	db SNP ID	HGVS names	dbPubMed	MAF (gnomAD)		
				EAS	SAS	EUR
ATM	rs139379666	NC_000011.9: g.108235879C>T	Conflicting	4,01E−5	3,266E − 5	5,422E−5
	rs150757822	NC_000011.9: g.108183194A>C	VUS	0	6,539E−5	2,248E−4
	rs1800058	NC_000011.9: g.108160350C>T	VUS	0	6,246E−3	1,856E−2
BRCA2	rs80359254	NC_000013.10: g.32972584A>G	VUS	0	0	2,641E−5
MSH6	rs61756469	NC_000002.11: g.48010479C>T	VUS	8,372E−4	2,666E−4	4,203E−5
	rs142254875	NC_000002.11: g.48030603C>G	VUS	0	0	8,796E−6
MLH1	rs104894994	NC_000003.11: g.37035032C>T	Conflicting	0	6,533E−5	9,676E−4
	rs4986984	NC_000003.11: g.37053562C>T	VUS	4,411E−3	1,960E−5	1,552E−4
	rs367654552	NC_000003.11: g.37035090C>G	VUS	5,437E−5	0	0
PTEN	rs786201044	NC_000010.10: g.89692922T>C	HP	–	–	–
RAD51D	rs137886232	NC_000017.10: g.33428366G>A	HP	–	6,533E−5	–

SNP single nucleotide polymorphism, *VUS* variants of unknown significance, *HP* highly pathogenic, *MAF* minor allele frequency, *gnomAD* the Genome Aggregation Database, *EAS* East Asian, *EUR* European, *SAS* South Asian

somatic mutations, COSM4721157 and COSM5096, respectively.

In 54% of the patients, no clinically significant variants were identified, probably due to some limitations to our study. In particular, we used a panel of only 27 genes that did not include other BC-predisposing genes, such as *BLM, ESR1, FANCA* and *NQO2*.

In this study, over 20% of the Buryat BC patients were found to carry a rare VUS. Buryats are characterized by molecular diversity due to the long generation time or the mixed nature of origin compared with other ethnic groups living in Siberia [32–35]. It is obvious that a more detailed genetic analysis of the Buryats is required.

Conclusion

In this study, we provide the first description of two pathogenic germline variants in the *RAD51D* (rs137886232) and *PTEN* (rs786201044) genes in *BRCA1/2*-negative Mongoloid (Buryat) women with BC.

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Author contributions PG, AM, AS, AK, LP recruited patients, collected samples and conducted experiments. PG wrote the manuscript; and NB revised the manuscript. EC, NC, YT and LP supervised the project. All authors reviewed and approved the manuscript.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Compliance with ethical standards

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

Ethical approval The current study was approved by the review board of the Cancer Research Institute, and written informed consent was obtained from all patients and in compliance with the recommendations of the Helsinki Declaration.

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