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Mutations in exon region of BRCA1-related RING domain 1 gene and risk of breast cancer

Jun Wu[1] | **Alibiati Aini[1]** | **Binlin Ma[2]** ⓘ

[1]Department of Head and Neck Surgery, Affiliated Tumor Hospital, Xinjiang Medical University, Urumqi, P.R. China

[2]Department of Breast and Thyroid Surgery, Affiliated Tumor Hospital, Xinjiang Medical University, Urumqi, P.R. China

Correspondence
Binlin Ma, Department of Breast and Thyroid Surgery, Affiliated Tumor Hospital, Xinjiang Medical University, No. 789 Suzhou East Street, Xinshi District, Urumqi 830011, P.R. China.
Email: Mabinlin123@126.com; mbldoctor@126.com

Abstract

Background: BRCA1-associated RING Domain 1 (BARD1) is an important gene related to breast cancer development. However, the role of BARD1 mutations in breast cancer remains inconclusive. This study is to investigate the relationship between exon mutations of BARD1 gene and the risk of early-onset breast cancer.

Methods: Totally, 60 cases of early-onset breast cancer patients (age 30–40 years) and 240 healthy women (age 30–40 years) were enrolled. Exon mutations of BARD1 were detected and analyzed by direct sequencing and SNaPshot.

Results: The risk of breast cancer was increased by 3.475 times in carriers with deletion mutation at rs28997575 site of BARD1 ($aOR_1 = 3.475$, 95%CI = 1.302–9.276) ($p = 0.013$). The risk of breast cancer in carriers with GC genotype at rs2229571 site of BARD1 was reduced by 72.6% ($aOR_1 = 0.274$, 95%CI = 0.134–0.562) ($p = 0.001$), and that in carriers with CC genotype was reduced by 82.8% ($aOR_1 = 0.172$, 95%CI = 0.076–0.392) ($p = 0.001$). After stratification with family history, the difference of rs2229571 site mutation genotype was statistically significant (OR = −2.169, 95%CI = 0.016–0.828, $p = 0.032$). Additionally, the frequency distribution of breast cancer family history in the case group (15%) was significantly more than that in the control group (6.7%) ($p = 0.037$).

Conclusion: The deletion mutation at rs28997575 locus of the BARD1 gene can significantly increase the risk of breast cancer. The mutation genotype of rs2229571 locus can significantly reduce the risk of breast cancer. Family history is associated with BARD1 gene polymorphism. A family history of breast cancer may be a risk factor for breast cancer.

KEYWORDS
BARD1 gene, early-onset breast cancer, single nucleotide polymorphism

1 | BACKGROUND

Breast cancer is the most common female malignancy in the world (Gervas et al., 2019) and the fifth leading cause of death among women worldwide (Al-Wajeeh et al., 2020).

The breast cancer susceptibility gene (*BRCA*) is the main tumor-associated gene found in the breast cancer development (Cheng & Huang, 2018). About 5–10% of breast cancers are considered to be hereditary and are attributed to pathogenic mutations in the *BRCA1* and *BRCA2* genes

(Cybulski et al., 2019). The pathogenic variant of *BRAD1* (BRCA1-associated RING domain protein 1) (c.1509del) was found in hereditary breast cancer patients with negative BRCA1 and BRCA2 gene test results (Rodríguez-Balada et al., 2019). *BARD1* gene is located on chromosome 2 q34-35, similar in sequence and structure to the *BRCA1* gene. The ring finger functional region of BRAD1 at the amino-terminal can combine with the zinc finger domain of the BRCA1 (Densham et al., 2016). *BARD1* is considered as a new tumor candidate gene of breast cancer patients without hereditary *BRCA1* and *BRCA2* gene mutations (Liu et al., 2017). A preliminary study found that the expression of BARD1 variants in breast cancer tissues was abnormally higher than that in paracancerous tissues and normal breast tissues, and it was related to the poor prognosis of breast cancer patients (Hua et al., 2012).

BARD1 and BRCA1 can form heterodimers through their N-terminal ring finger domains. This interaction is essential for the stability of BRCA1. BRCA1/BARD1 heterodimers have E3 ubiquitin ligase activity and can promote ubiquitination of RNA polymerase II, thus preventing DNA transcription damage and restoring the genetic stability (Cimmino et al., 2020). BRCA1/BARD1 heterodimers can also interact with Obg like ATPase 1 and regulate the number of centrosomes to maintain genomic integrity and thus inhibit tumor progression (Yoshino et al., 2018). In contrast, inhibition of the interaction between BARD1 and BRCA1 can promote tumorigenesis (Kim, Ha, Campo & Breuer, 2018). Therefore, the structure and function of BARD1 are strictly dependent on the complete heterodimer formation. Once BARD1 is mutated, the mutant BRCA1-BARD1 complex is not able to bind to nucleosomes, which produces histone H2A ubiquitination defects, leading to an increased risk of breast cancer (Stewart et al., 2018).

Therefore, we designed a case-control study. Blood samples were collected from breast cancer patients and healthy people. The mutations in the exon of BRAD1 gene were detected by SNaPshot typing, which is an important method for analyzing genetic variation (Koshy et al., 2017). Their pathogenicity was analyzed to further understand the association between BRAD1 gene and breast cancer risk.

2 | METHODS AND MATERIALS

2.1 | Subjects

This study enrolled 60 cases of Han women with early-onset breast cancer from the Affiliated Tumor Hospital of Xinjiang Medical University between 2014 and 2019. They were aged 30–40 years. For control, 240 age-matched healthy Han women who received physical examination in the same hospital during the same period were also enrolled. They were

healthy volunteers and had no history of systemic diseases or any other diseases. Detailed clinical information was collected from all subjects. Peripheral blood (5 mL) was collected from each subject. All enrolled patients have signed informed consent. This study was approved by the Ethics Committee of Xinjiang Medical University Cancer Hospital.

Inclusion criteria for breast cancer patients: (a) patients were with early-onset breast cancer confirmed by pathological diagnosis; (b) patients did not receive radiotherapy or chemotherapy before surgery; (c) patients with complete clinical basic information. Exclusion criteria for breast cancer patients: patients with uncertain pathological diagnosis, incomplete medical records, hematological diseases, immune system diseases, or received anti-tumor treatment (such as neoadjuvant chemotherapy) before surgery, were excluded.

Inclusion criteria for control group: Healthy females of Han nationality with no related tumor history and no major disease. Exclusion criteria: (a) Age was not in the range of 30–40 years old; (b) basic personal information was incomplete; (C) subjects with hematological diseases or immune system diseases.

2.2 | DNA sequencing

Genomic DNA was extracted from peripheral blood using the routine phenol/chloroform method as previously described (Koshy et al., 2017). The DNA concentration and purity were measured by a Biophotometer (Eppendorf). Fourteen pairs of primers were designed to specifically amplify 26 fragments, covering 84 kb of the whole gene region using Primer3 (Howard Hughes Medical Institute National Institutes of Health), and synthesized by Shanghai Tianhao Biotechnology Co., Ltd. The PCR products were amplified, purified, and then sequenced using the ABI version 3.1 Big Dye kit (ABI, cat #4337456) and ABI 3130 Sequencer (ABI, 3130XLR). The sequencing results were analyzed by the Polyphred software (Dr. Deborah Nickerson's lab at the University of Washington in Seattle). With this software, the original sequencing data can be analyzed to obtain various mutation information in the target sequence fragments, and multiple samples can be compared and analyzed at the same time. All the primers are listed in Table 1.

2.3 | SNaPshot typing

The genotyping procedures were conducted as described previously (Tobler et al., 2005). Briefly, based on the principle of mutual substitution of tag sites, high-frequency sites were selected from the sequencing results for SNaPshot typing, including rs1048108, rs28997575, rs2229571, rs2070094, and rs3738888. For multiplex PCR reaction, primers were

TABLE 1 Primers used for gene sequencing

Fragment	Direction	Sequence
P1	Forward	TGATGCACCTGAGAGAATCCA
	Reverse	CATGTCTGAAAAGATCTGGTTTACTTTT
P2	Forward	CGGCGATCAGTGAAAGATCTGGAGGAG
	Reverse	GGGGCCGGACGGCTGAAACTT
P3	Forward	CTCTGTCCCCGGCGTGTTCTCG
	Reverse	CGCGGGAACGGAAGGAGGAAAC
P4	Forward	TATGGTAGTGTTGGGCCTTGG
	Reverse	TCCAATTTGGCAAAGCTGTCT
P5	Forward	TTCATAGCAAATTACATGAGCAACC
	Reverse	CGTATTCCAGAACTCCAGATAGATG
P6	Forward	AATTCTTCGGGAGCTCCATGT
	Reverse	TGGCCACGTTTTCCATTATTT
P7	Forward	GCCAACCATCTGTTATCTCCA
	Reverse	TGAATCTGGCTTCTCTGGTTCA
P8	Forward	CCACACCCGGCCTAATAATTT
	Reverse	TTTGGTTCCAGTGACATGCAG
P9	Forward	GACCCAAGGCTCAAATGAGAA
	Reverse	GCTGATTATGAGTGCAGAATGTGA
P10	Forward	TTCTCCTTTGAGTTCAGCAGCTT
	Reverse	TGGTAAGCTCCTCCCTGAGAA
P11	Forward	GGTGGAATAGGGAATTGCTGA
	Reverse	ACAGCCATCTCCCAATGGTTA
P12	Forward	TGTGTTTGCCAATATGGCTTT
	Reverse	GACAGGGCTTCACCGTGTTAG
P13	Forward	TGCCATGAAGAAGAAAAACCA
	Reverse	GCAATGTTCAAGATGCCAAAA
P14	Forward	CATGTGACAGGTCACGGTCAG
	Reverse	TCAAAGACAAATATGAATGACTCTACC

Note: For each fragment, the PCR fragment was sequenced with a PCR primer to guarantee that the overlapping regions of the sub-primers can be tested and that all 11 exon regions can be tested.

designed using Primer3 (Howard Hughes Medical Institute National Institutes of Health). HotStarTaq (Qiagen, Cat# 203203) was used for amplification. The PCR products were purified by shrimp alkaline phosphatase (Thermofisher, Cat# 783901000UN) and exonuclease I (Thermofisher, Cat# 720735KU). The products were then used for extension reactions using the SNaPshot Multiplex kit (ABI). The extension product was purified with shrimp alkaline phosphatase and sequenced by ABI3730 Sequencer (ABI). The SNP (single nucleotide polymorphism) typing was analyzed by GeneMapper4.1 (Applied Biosystems).

2.4 | Statistical analysis

SPSS 20.0 statistical software was used for statistical analysis. The χ^2 test was used to compare the differences of count data. The *t*-test was used to compare the differences of measurement data. Binary Logistic regression analysis was used to analyze the relationship of exon genotype of BARD1 with breast cancer susceptibility. The odds ratio (OR), and confidence interval (95% CI) were obtained. Two-sided test with $p < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Clinical data of breast cancer patients and control

The basic clinical data of breast cancer patients and control were shown in Table 2. We found that there were no significant differences in body mass index, age at menarche, age of first delivery, and number of pregnancies between the patient and the control group ($p > 0.05$) (Table 2). The percentage of family history of breast cancer in breast cancer

	Breast cancer group (N = 60)	Control group (N = 240)	p
Body mass index (mean ± SD)	23.02 ± 3.92	23.02 ± 3.65	0.963
Menarche age (mean ±SD)	13.28 ± 1.59	12.98 ± 1.28	0.099
Age of first delivery (mean ± SD)	27.26 ± 3.77	27.34 ± 3.24	0.863
Number of pregnancies			
0–1 times	24 (40.0)	98 (40.8)	0.906
≥2 times	36 (60.0)	142 (59.2)	
Family history of breast cancer			
Yes	9 (15.0)	16 (6.7)	0.037
No	51 (85.00)	224 (93.3)	

Note: χ^2 test was used to compare the differences in the number of pregnancies and the family history of breast cancer between the patient and control group. t-test was used to compare the differences in body mass index, age of menarche, and age of first delivery between patient and control group.

TABLE 3 BARD1 gene exon and promoter region mutation results

SNP sites in breast cancer group	SNP sites in control group	Nucleotide substitution	Location	Amino acid	Type of mutation
rs1048108	rs1048108	c.70C > T	EXON1	Pro24Ser	M
rs2070096	—	c.1053G > C	EXON4	Thr351Thr	S
rs28997575	rs28997575	c.1075_1095del	EXON4	Leu359_Pro365del	Del
rs2229571	rs2229571	c.1134G > C	EXON4	Arg378Ser	M
rs2070093	—	c.1518T > C	EXON6	His506His	S
rs2070094	rs2070094	c.1519G > A	EXON6	Val507Met	M
rs3738888	rs3738888	c.1972C > T	EXON10	Arg658Cys	M

Note: (1) For the 60 cases of blood samples of breast cancer, the first-order sequencing method was used to sequence the exons and promoter regions of the BARD1 gene. (2) According to the principle of mutual substitution of tag sites, the high frequency sites were selected from the breast cancer groups. The SNaPshot typing was used to detect the above sites in 240 healthy female blood samples.

Abbreviations: Del, deletion mutation; M, missense mutation; S, synonymous mutation.

patients (15%) was significantly higher than that in control group (6.7%) (p = 0.037). This suggests that family history of breast cancer may be a risk factor for breast cancer.

3.2 | The sequencing results

A total of 7 mutation sites were detected in the breast cancer patient group, including 4 missense mutations, 2 synonymous mutation, and 1 deletion mutation (Table 3). Five sites of high-frequency were screened out and further verified in the control group, which were located at EXON1, EXON4, EXON6, and EXON10 (Table 3).

3.3 | Distribution of genotypes of BARD1 exon in breast cancer and control groups

We next compared the distribution of genotypes of BARD1 exon in breast cancer and control groups. We found a deletion at the rs28997575 site. The frequency of this deletion

mutation in the breast cancer group (13.3%) was significantly higher than that in the control group (4.2%) (χ^2 = 5.618, p = 0.013). There was also a missense mutation at rs2229571, and the frequency of GC type, CC type, and GC +CC dominant model at rs2229571 site were significantly different between breast cancer group and control group (χ^2 = 5.872, p = 0.015; χ^2 = 7.654, p = 0.006, χ^2 = 8.398, p = 0.004). Additionally, a missense mutation was found at the rs2070094 site. The GA type and GA+AA dominant model frequency were significantly different between the breast cancer group and the control group (χ^2 = 8.384, p = 0.004; χ^2 = 5.357, p = 0.021) (Table 4). These results indicate that the above three loci may be related to the incidence of breast cancer.

3.4 | Logistic regression analysis of genotype of BARD1 exon and breast cancer susceptibility

To further explore the relationship of the exon genotype of BARD1 gene and breast cancer susceptibility, binary

TABLE 4 Distribution of BARD1 exon genotype in breast cancer group and control group (%)

Polymorphism	Breast cancer group (N = 60)	Control group (N = 240)	χ^2	p
rs1048108				
CC	24 (40.0%)	101(42.1%)	—	—
CT	29 (48.3%)	102 (42.5%)	0.336	0.562
TT	7 (11.7%)	37 (15.4%)	0.235	0.628
CT+TT	36 (60.0%)	139 (57.9%)	0.086	0.770
rs28997575				
Wild-type	52 (87.7%)	230 (95.8%)	—	—
Mutation	8 (13.3%)	10 (4.2%)	5.618	0.013
rs2229571				
GG	18 (30.0%)	34 (14.2%)	—	—
GC	25 (41.7%)	113 (47.0%)	5.872	0.015
CC	17 (28.3%)	93 (38.8%)	7.654	0.006
GC+CC	42 (70.0%)	206 (85.8%)	8.398	0.004
rs2070094				
GG	36 (60.0%)	104 (43.3%)	—	—
GA	14 (23.3%)	107 (44.6%)	8.384	0.004
AA	10 (16.7%)	29 (12.1%)	0.000	0.993
GA+AA	24 (40.0%)	136 (56.7%)	5.357	0.021
rs3738888				
CC	57 (95.0%)	231 (96.3%)	—	—
CT	3 (5.0%)	9 (3.7%)	0.005	0.712

Note: The χ^2 test was used to compare the differences in the frequency of BARD1 genotype loci between patient and control group. Two-sided test with $p < 0.05$ was considered statistically significant.

Logistic regression was carried out. We found that the carriers of the rs28997575 deletion genotype increased the risk of breast cancer (Table 5). The rs1048108, rs2070094, and rs3738888 loci were not found to be associated with the risk of breast cancer ($p > 0.05$). Carriers of the GC genotype at rs2229571 site showed reduced risk of breast cancer. After further adjusting for the family history, we found that the rs28997575 deletion genotype carriers resulted in a 3.475-fold significantly increased risk of breast cancer compared with wild genotype carriers (aOR1 = 3.475, 95% CI = 1.302–9.276, $p = 0.013$) (Table 6). For the rs2229571 locus, compared with GG genotype carriers, GC genotype carriers reduced breast cancer risk by 72.6% (aOR1 = 0.274, 95% CI = 0.134–0.562, $p = 0.001$). CC genotype carriers reduced breast cancer risk by 82.8% (aOR1 = 0.172, 95% CI = 0.076–0.392, $p = 0.001$). In Table 7, the "family history" was stratified. Compared with the GG wild genotype carrier at rs2229571 locus, the aOR1 of mutant genotype (GC+CC) was 0.114 and 95% CI was 0.016–0.828, which was significant ($p = 0.032$). All of these results suggest that the genotype of rs28997575 locus deletion mutation in BARD1 gene significantly increases the risk of breast cancer whereas the missense genotype of rs2229571 locus significantly reduces the risk of breast cancer. More importantly, family history and BARD1 gene polymorphism are related.

4 | DISCUSSION

Breast cancer is a common disease that affects more than 1 million women worldwide every year (Bray et al., 2018). About 5%–10% of breast cancer cases are hereditary, but this percentage depends on the study population and the genes being evaluated (Cybulski et al., 2019). If mutation investigation is limited to familial cases, the proportion will be higher (Cybulski et al., 2019). Risk models of different subtypes of breast cancer predict that the susceptibility genes (BRCA1, BRCA2, BARD1, RAD51D and PALB2) have a lifetime risk of breast cancer greater than 20% (Shimelis et al., 2018). BARD1 gene was identified in 1996 in an effort to understand the biological function of BRCA1 protein. Although potentially pathogenic BARD1 variants have been reported (Alenezi, Fierheller, Recio & Tonin, 2020), the role of BARD1 in cancer predisposition remains inconsistent. A recent study used a panel of 34 putative susceptibility genes to perform sequencing on samples from 60,466 women with breast cancer and

Polymorphism	β	SE (β)	Wals	p	OR	95% CI
rs1048108						
CC					1.000	
CT	0.182	0.313	0.339	0.561	1.200	0.650–2.215
TT	−0.135	0.452	0.089	0.766	0.874	0.360–2.120
CT+TT	0.105	0.296	0.125	0.724	1.110	0.621–1.984
rs28997575						
Wild-type					1.000	
Mutation	1.401	0.485	8.349	0.004	4.059	1.569–10.498
rs2229571						
GG					1.000	
GC	−1.081	0.351	9.514	0.002	0.339	0.171–0.674
CC	−1.631	0.412	15.660	0.000	0.196	0.087–0.439
GC+CC	−1.290	0.327	15.558	0.000	0.275	0.145–0.523
rs2070094						
GG					1.000	
GA	−0.027	0.312	0.008	0.931	0.973	0.528–1.794
AA	0.236	0.442	0.258	0.593	1.266	0.532–3.011
GA+AA	0.034	0.293	0.014	0.907	1.035	0.583–1.836
rs3738888						
CC					1.000	
CT	−0.423	0.693	0.372	0.542	1.526	0.392–5.936

Note: Binary Loostic regression was used to analyze the genotype of the BARD1 gene exon region, breast cancer susceptibility, aOR1, and confidence interval (95% CI). Two-sided test with $p < 0.05$ was considered statistically significant.

53,461 controls (Dorling et al., 2021). They showed that protein-truncating variants in 4 genes (BARD1, RAD51C, RAD51D, and TP53) were associated with a risk of breast cancer. Another study reported that the overexpression of the oncogenic isoforms BARD1β and BARD1δ could permit cancer development, indicating that the BARD 1 gene offers new hope for improving breast cancer therapy (Krzeszowiec, Kmieć & Wydra, 2020). Therefore, BARD1 is an important gene related to the development of breast cancer.

In this study, it was found that the rs28997575 site (c.1075_1095del) was a deletion mutation (Leu359_Pro365del). After adjusting for family history, the risk of breast cancer in carriers with genotype deletion at this site increased by 3.475 times. More importantly, a potentially pathogenic deletion mutation was also found in a previous study (Rodríguez-Balada et al., 2019), which identified 25 genetic mutations in 77 patients with hereditary cancer who tested negative for the BRCA1 and BRCA2 gene mutations. Nine mutations were found in seven different genes and confirmed by sequencing. Six variants were classified as pathogenic or likely pathogenic, three of which were in the PALB2 gene, BRIP1 (BRCA1 interacting protein

C-terminal helicase 1) gene, BARD1 gene (c.1509del) and RAD50 gene. These findings are consistent with the previous study on hereditary breast cancer (Moran et al., 2017). Deletion mutations are those in which a codon encoding an amino acid is deleted by a base and become a codon encoding another amino acid, thereby changing the amino acid type and sequence of the polypeptide chain and causing changes in the translated protein. Therefore, deletion mutations are more likely to cause breast cancer pathogenicity.

It is reported that the BARD1 gene rs2070094 (Val507Met), rs2229571 (Arg378Ser), and rs1048108 (Pro24Ser) mutations are located in the region where BARDl enhances heterodimer ubiquitin ligase activity (Xia, Pao, Chen, Verma & Hunter, 2003). These polymorphism mutations may affect the heterodimer ubiquitin ligase activity, which in turn affects the functions of BARD1-BRCA1 heterodimers such as cell cycle regulation, transcription regulation, and DNA repair (Xia et al., 2003). In this study, a missense mutation was found at the rs2070094 (Val507Met) locus. The GA type and GA+AA dominant model frequency were significantly different between the breast cancer group and the control group.

TABLE 6 Logistic regression analysis of the BARD1 exon genotype and breast cancer susceptibility after adjusting for family history

Polymorphism	β	SE (β)	Wals	p	aOR1	95% CI
rs1048108						
CC					1.000	
CT	0.032	0.325	0.977	0.323	1.379	0.729–2.610
TT	−0.004	0.461	0.000	0.993	0.996	0.404–2.45
CT+TT	0.241	0.309	0.610	0.435	1.273	0.695–2.332
rs28997575						
Wild-type					1.000	
Mutation	1.246	0.501	6.184	0.013	3.475	1.302–9.276
rs2229571						
GG					1.000	
GC	−1.294	0.367	12.457	0.000	0.274	0.134–0.562
CC	−1.758	0.420	17.556	0.000	0.172	0.076–0.392
GC+CC	−1.480	0.340	18.956	0.000	0.228	0.117–0.443
rs2070094						
GG					1.000	
GA	−0.182	0.328	0.307	0.580	0.834	0.438–1.586
AA	0.043	0.461	0.009	0.926	1.043	0.423–2.577
GA+AA	−0.132	0.311	0.179	0.672	0.877	0.477–1.612
rs3738888						
CC					1.000	
CT	−0.049	0.753	0.004	0.948	0.952	0.218–4.167

Note: The adjusted variable was family history. After adjusting for family history, binary Logistic regression was used to analyze the BARD1 exon genotype and breast cancer susceptibility as well as aOR1 and confidence interval (95% CI). Two-sided test with $p < 0.05$ was statistically significant.

After adjusting for family history, although a GA mutation at this site had a protective effect on the risk of breast cancer (aOR1 = 0.834), there was no statistical significance. The genotype frequency of rs1048108 (Pro24Ser) locus was not significantly different between breast cancer group and control group. A missense mutation was also observed at the rs2229571 (Arg378Ser) locus, and GC and CC genotypes at rs2229571 played a protective role in the risk of breast cancer (aOR1 = 0.274; aOR1 = 0.172). We further stratified the population with "family history" and found that GC+CC genotype at rs2229571 played a significant protective role in the risk of breast cancer (aOR1 = 0.114). Our research thereby suggests that the rs2229571 of BARD1 significantly reduces the risk of

breast cancer, and there is a correlation between family history and BARD1 gene polymorphisms. Ishitobi M et al. (Ishitobi et al., 2003) also conducted a study on 73 premenopausal and 70 postmenopausal breast cancer patients and 155 healthy controls in the Japanese population and found that the rs2229571 (Arg378ser) locus was not associated with breast cancer susceptibility. Onay et al. (2006) studied 398 breast cancer patients and 372 healthy controls in the Canadian population and found that both the CT and TT genotype of rs1048108 (Pro24ser) site could reduce the risk of breast cancer by 20% (95% CI = 0.6–1.1 and 0.5–1.2), however, there was no significant difference. These findings indicate that the mutations at rs2070094 (Val507Met), rs2229571 (Arg378Ser), and rs1048108

TABLE 7 Logistic regression analysis of genotypes of rs28997575 and rs2229571 loci and breast cancer susceptibility after stratification of family history

Family history	Polymorphism	β	SE (β)	Wals	p	aOR1	95% CI
Yes	rs28997575						
	Wild-type					1.000	
	Mutation	0.405	0.913	0.197	0.657	1.500	0.251–8.977
No	rs28997575						
	Wild-type					1.000	
	Mutation	1.077	0.593	3.299	0.069	2.935	0.918–9.379
Yes	rs2229571						
	GG					1.000	
	GC+CC	−2.169	1.011	4.606	0.032	0.114	0.016–0.828
No	rs2229571						
	GG					1.000	
	GC+CC	−0.719	0.374	3.702	0.054	0.487	0.234–1.014

Note: After further stratifying the family history, binary Logistic regression analysis was used to test the genotype of BARD1 gene exon region genotype and breast cancer susceptibility aOR1, and confidence interval (95% CI). Two-sided test with $p < 0.05$ was considered statistically significant.

(Pro24Ser) mainly have protective roles, but the results are not consistent. The reason may be that: (a) the carcinogenic mechanism of the BARD1 gene is complicated and may be affected by other factors; (b) there may be regional differences, small samples, or systematic errors of different genotyping methods. Therefore, large-scale research with large sample size is needed.

As of March 31, 2021, the dbSNP and ClinVar databases have reported that rs28997575 is benign or possibly benign, but not malignant. Similarly, the lasted data in the dbSNP and ClinVar databases have shown that the rs2229571 is a benign mutation. The rs28997575 or rs2229571 was not found in the gnomAD database. However, our study found that the BARD1 gene rs28997575 locus deletion mutation may significantly increase the risk of breast cancer whereas the rs2229571 locus mutation may significantly reduce the risk of breast cancer. This difference in rs28997575 may be caused by ethnic differences or regional differences. Further studies are needed to verify our results.

In summary, there is a correlation between BARD1 gene and breast cancer. Mutations at different sites in the exon of the BARD1 gene have an impact on the risk of breast cancer. Deletion mutation at rs28997575 site of BARD1 gene can significantly increase the risk of breast cancer, whereas missense mutation at rs2229571 site can reduce the risk of breast cancer. Also, family history and BARD1 gene polymorphism are related.

ORCID
Binlin Ma https://orcid.org/0000-0002-5432-9632

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