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ORIGINAL ARTICLE

Two tSNPs in BRIP1 are associated with breast cancer during TDT analysis

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Abstract

Objectives: This study aimed to investigate and confirm the association between 15 single nucleotide polymorphisms of four susceptibility genes (*NBS1*, *TP53*, *PTEN*, and *BRIP1*) and the susceptibility of breast cancer.

Methods: The genome DNA was extracted from peripheral blood and tumor tissues from one hundred and seventeen core families. 15 SNPs were detected by PCR. The transmission disequilibrium test (TDT) and the Hardy–Weinberg equilibrium (HWE) are used to verify the association between these SNPs and breast cancer. Further correlation between SNPs and certain pathological features of the tumor, including tumor size, location of lymph nodes, pathologic classification, and the stage and subtype of breast cancer, are analyzed by the chi-square test and logistic regression analysis.

Results: Based on TDTs, two SNPs of rs7220719 and rs11871753 in *BRIP1* showed a significant association with breast cancer, while the other 13 selected SNPs did not. However, further statistical analysis demonstrated no obvious differentiation in the clinical characteristics of breast cancer between 37 patients with rs7220719 and 80 patients with wild types. Similar results were also found for rs11871753.

Conclusions: The data provided the evidence for the association between two SNPs of *BRIP1* and breast cancer, but did not affect certain clinical phenotypes.

KEYWORDS

breast cancer, *BRIP1*, SNPs, transmission disequilibrium test

1 | INTRODUCTION

Breast cancer, which is one of the most common malignant tumors in the world, is the second leading cause of cancer death among women. China has the most breast cancer patients in the world in 2014 (Liang et al., 2019). The causes of breast cancer include lifestyle, environment, and hereditary cause (Sun et al., 2017). Previous studies have confirmed that breast cancer has a genetic susceptibility associated with SNP polymorphism, and genetic mutations in FANCD2 pathway, which are closely related to the occurrence of breast cancer (Cox et al., 2018; Zanna et al., 2018).

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The main function of FANCD2 pathway is to repair DNA interstrand crosslinks (ICLs) with several other DNA repair proteins by nucleotide excision repair (NER) and homologous recombination (HR) and preserve genomic integrity (Niraj et al., 2019). Several important genes related to FANCD2 pathway, including *BRCA1* (OMIM: 113705), *BRCA2* (OMIM: 600185), *RAD51* (OMIM: 179617), *PALB2* (OMIM: 610355), *NBS1* (OMIM: 602667), *TP53* (OMIM: 191170), *PTEN* (OMIM: 158350), and *BRIP1* (OMIM: 605882), have been reported to play synergistic effect on DNA repair. FANCD2 usually is activated by FA proteins and translocates to damage-induced nuclear foci containing BRCA1, BRCA2, and RAD51, repairing DNA interstrand crosslinks (Shahi et al., 2019). PTEN binds to the RAD51 promoter to regulate its transcription. BRCA2 and BRIP1 are downstream of the FANCD2 activation step. FANCD2 also interacts with the MRE11–NBS1–RAD50 complex to prevent genomic instability and repair DNA double-strand breaks (Walsh & King, 2007; Kleibl and Kristensen, 2016; Bai et al., 2019), while FANCF is able to increase the expression of TP53, which can affect cell transformation and proliferation (D'Andrea & Grompe, 2003; Silwal-Pandit et al., 2017; Schon and Tischkowitz, 2018).

Some of these genes have been extensively reported to associate with various tumors. More and more evidence supported that the genetic variations, such as pathogenic mutations and SNPs, in FANCD2 pathway-related genes, play a very important role in breast cancer, especially for *BRCA1* and *BRCA2* (Nalepa & Clapp, 2018). Based on the previous case–control research, we also find the correlation between the risk of breast cancer occurrence and some SNPs in *NBS1*, *TP53*, *PTEN*, and *BRIP1* genes, including rs1042522, rs2299941, rs2735385, rs6999227, rs1805812, rs1061302, rs1042522, rs2735343, rs7220719, rs16945628, and rs11871753. Some reports have demonstrated that rs1061302 and rs2735343 have been also analyzed in other cancers such as lung and upper aerodigestive tract (UADT) cancers, systemic lupus erythematosus, and esophageal squamous carcinoma. Although other studies analyzed these SNPs, they have not been discussed in breast cancer by TDT analysis among core families.

Thus, in this study, we selected 15 tag SNPs of breast cancer susceptibility genes, including rs192236678, rs146605798, rs72550742, rs182030463, rs147494981, rs182756889, rs2735385, rs6999227, rs1805812, and rs1061302 (NBS1); rs1042522 (TP53); rs2735343 and rs2299941 (PTEN); and rs7220719, rs16945628, and rs11871753 (BRIP1), and detected through TDT analysis among one hundred and seventeen core families. Further correlation between different clinical features and SNPs was also determined.

2 | METHODS AND MATERIALS

2.1 | Study population

This study was approved by the breast center of Xiangya Hospital Central South University in China. This research obtained ethical approval, and written informed consent was obtained from all participants. One hundred and seventeen families including four hundred and forty-two samples were recruited in the Department of Breast Surgery of Xiangya Hospital and were divided into case group and control group. The subjects of the study were all Chinese Han people, the parents of the patients were all randomly married, and there was no blood relationship between these families. All the families are core families that include patients and their parents, and some affect their brothers or sisters. All the patients were diagnosed with pathology, and their parents were healthy and had no history of special diseases. Clinical information was collected including the size of tumor, the location of lymph nodes, pathologic diagnosis, and the stage and subtype of breast cancer (Ma et al., 2013).

3 | GENOTYPING

Four genes: *NBS1* (RefSeq: NC_000008.11), *TP53* (RefSeq: NC_000017.11), *PTEN* (RefSeq: NC_000010.11), and *BRIP1* (RefSeq: NC_000017.11) are included in the study, which reference GRCh38.p13 Primary Assembly. We collected the peripheral blood DNA and tumor tissue of all members of the case group and the control group. 5 ml of anticoagulated whole blood was taken, and DNA was extracted using kit and then quantified with UV spectrophotometer and diluted to 80 μg/ml. PCR system was 50 μl, and 80 ng DNA template was added to each tube. Common reverse primer (10 μm) 1 μl, mutation-specific forward primer (10 μM) 1 μl, or wild-type forward primer (10 μM) 1 μl, 10 × PCR buffer 5 μl, 25 mM MgCl 23 μL, 10 mM dNTPs 1 μl, and 5 Uμl Taq polymerase 1μL with deionized water were added and placed in the MJ Research PTC-100 Gene Amplification Instrument according to the following procedure: first 94°c denaturation for 11 min, and then the amplification cycle, including denaturation for 40 s (94°c), annealing for 1 min (54°c), and extension for 1 min (72°c). 35 cycles were amplified and finally extended for 10 min (72°c). AS-PCR products were identified by 2.0% agarose gel (containing EB) electrophoresis (Zhang et al., 2014).

4 | STATISTICAL METHODS

We used the Hardy–Weinberg equilibrium (HWE) and family-based transmission disequilibrium test (TDT) implemented

by Shanghai Genesky Biotechnologies Company (software: plink 1.9, https://www.cog-genomics.org/plink/1.9/). In the TDT, we can consider the gene transitive relationship between patients and their parents. A P value equal to or less than 0.05 was considered statistically significant. Then, we classified the patient's pathological information according to international standards and analyzed the relationship between the two SNPs and this information using the chi-square test and logistic regression analysis by SPSS software.

5 | RESULTS

5.1 | Two SNPs in *BRIP1* were associated with breast cancer by TDT analysis

A total of one hundred and seventeen families were involved in the analysis. Table 1 shows that the rs1042522 did not satisfy the HWE and thus be excluded before the TDT ($p < 0.05$). According to the result of TDT, two polymorphisms in *BRIP1* gene were found to be significant to breast cancer ($p < 0.05$), and the other thirteen polymorphisms did not satisfy the TDT (Table 2). The result indicated that rs7220719 ($p = 0.03197$) and rs11871753 ($p = 0.00971$) of BRIP1 gene are related to breast cancer. As rs7220719 and rs11871753 were located in the intron, their functions need to be further investigated. The other thirteen SNPs did not show the relationship of breast cancer during the TDT analysis ($p > 0.05$).

TABLE 1 The Hardy–Weinberg equilibrium of breast cancer patients

SNP	AFF MAF	AFF HWE	UNAFF MAF	UNAFF HWE
rs1042522	0.4171	0.0321	0.4534	0.2931
rs1061302	0.4415	0.3215	0.4089	0.4191
rs11871753	0.1512	0.5835	0.178	0.8238
rs146605798	0.0049	1	0.0042	1
rs147494981	0.0122	1	0.0042	1
rs16945628	0.3659	0.7636	0.4025	0.4984
rs1805812	0.1268	1	0.1165	0.7495
rs182030463	0	1	0	1
rs182756889	0	1	0	1
rs192236678	0.0024	1	0.0042	1
rs2299941	0.2951	1	0.2987	0.8765
rs2735343	0.4902	0.3278	0.4831	1
rs2735385	0.3976	0.3822	0.3856	0.5851
rs6999227	0.4146	0.7747	0.4004	0.5879
rs7220719	0.1537	0.793	0.1801	1
rs72550742	0.0195	1	0.0127	1

Abbreviations: AFF, affected; HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency.

5.2 | SNPs rs7220719 and rs11871753 did not associate with the clinical phenotype

Then, we divided these patients into several groups according to patients' size of tumor, location of lymph nodes, pathologic diagnosis, and the stage and subtype of breast cancer and analyzed the association between the mutation and these clinical characteristics. The information-unknown patients are divided into a separate group. We divided the patients into three groups by the size of tumor: smaller than 2 cm, 2 cm to 5 cm, and larger than 5 cm. The lymph nodes are also considered in the grouping. We also divided the patients by the number of lymph metastasis: 0, 1–3, and more than 3. The patients' subtype and stage are according to international standard. The detailed grouping is shown in Tables S2 and S3. However, the result of chi-square test and logistic regression analysis demonstrates no obvious difference between the mutation group and the control group (Tables 3–6) (Huo et al., 2009; Sun, Zhao, et al., 2017; Vahednia et al., 2019).

6 | DISCUSSION

This study used transmission disequilibrium test to analyze the influence of 15 SNPs among core families, which is the most rigorous method. For familial genetic diseases, individuals of different generations have genetic relationships, and disease-related loci are passed from father to offspring. The TDT takes this transitive relationship into account. One hundred and seventeen core families are a large sample size for transmission disequilibrium test; thus, we can obtain a more rigorous result. We also analyzed the clinical features of patients to make further analysis of the role of these SNPs to enhance experimental integrity.

The breast cancer is a complex multifactorial disease and may result from the interaction between protective and predisposing genomic variants and the infection of environmental factors. In the present study, the association between breast cancer and *NBS1*, *TP53*, *PTEN*, and *BRIP1* genes was investigated.

The tSNPs are selected based on other's studies and the NCBI database and may have synergistic action involved in common pathway. In our previous study, we also found that rs2299941, rs2735385, rs6999227, rs1805812, rs1061302, rs1042522, rs2735343, rs7220719, rs16945628, and rs11871753 may be associated with the risk of breast cancer; thus, the TDT analysis is needed to verify the association. We selected these fifteen SNPs in our studies (rs192236678, rs146605798, rs72550742, rs182030463, rs147494981,

TABLE 2 The transmission disequilibrium test of breast cancer patients

SNP	A1	A2	T	U	OR	L95	U95	CHISQ	*p*
rs147494981	G	A	4	3	1.333	0.2984	5.957	0.1429	0.7055
rs2735385	A	C	96	79	1.215	0.9023	1.637	1.651	0.1988
rs6999227	C	G	101	86	1.174	0.8809	1.566	1.203	0.2727
rs1061302	C	T	111	86	1.291	0.974	1.71	3.173	0.07488
rs1805812	C	T	35	40	0.875	0.5559	1.377	0.3333	0.5637
rs192236678	T	G	1	1	1	0.06255	15.99	0	1
rs72550742	T	C	6	5	1.2	0.3662	3.932	0.09091	0.763
rs182030463	0	A	0	0	NA	NA	NA	NA	NA
rs146605798	A	G	2	2	1	0.1409	7.099	0	1
rs182756889	0	G	0	0	NA	NA	NA	NA	NA
rs2299941	G	A	89	88	1.011	0.7533	1.358	0.00565	0.9401
rs2735343	C	G	103	98	1.051	0.7971	1.386	0.1244	0.7243
rs1042522	G	C	86	120	0.7167	0.5433	0.9453	5.612	0.01784
rs7220719	A	G	46	69	0.6667	0.4591	0.9681	4.6	0.03197
rs11871753	A	G	41	68	0.6029	0.4092	0.8883	6.688	0.00971
rs16945628	T	C	77	102	0.7549	0.5615	1.015	3.492	0.06168

Abbreviations: A1, alleles with lower frequencies; A2, alleles with higher frequencies; CHISQ, chi-square statistics of TDT; T, number of low-frequency alleles inherited; U, number of low-frequency alleles that are not inherited.

rs182756889, rs2735385, rs6999227, rs1805812, and rs1061302 in *NBS1*; rs1042522 in *TP53*; rs2735343 and rs2299941 in *PTEN*; and rs7220719, rs16945628, and rs11871753 in *BRIP1*). The information of associated SNPs and their corresponding genetic information are shown in Table S1.

In this study, we evaluated the association of 2 common polymorphisms (rs7220719 and rs11871753) in *BRIP1*. As far as we know, these two related SNPs have not been studied by others. We found a statistically significant association between rs7220719 and rs11871753 and the risk of breast cancer. These two SNPs locate in the *BRIP1* gene's intron domain, and their functions are still unknown. BRIP1 is BRCA1-interacting protein, which can form a complex with the BRCT domain of BRCA1 in order to repair the double-stranded DNA breaks. It is essential for DNA repair pathways and plays the critical role of the BRCA–FA pathway in tumor development and progression (Hu et al., 2010; Ma, Cai, et al., 2013). This result deeply confirmed our previous research in 2012 among 734 Chinese women with breast cancer and 672 age-matched healthy controls. According to our study, rs7220719 had significant associations with breast cancer under the codominant model in unselected cases or familial and early-onset cases. The association did not exist under the dominant model and sporadic cases. rs11871753 was the same as rs7220719 in familial and early-onset cases, but it did not have significant association in unselected cases and the dominant model (Chen et al., 2018).

Although rs7220719 and rs11871753 are associated with the susceptibility of breast cancer, the loci analyzed in the clinical data did not show the affection of patients' clinical features, such as size of tumor, the location of lymph nodes, pathologic diagnosis, and the stage and subtype of breast cancer. In the next step, we will supplement the samples and carry out the functional study of the two loci to clarify its special role in the occurrence and development of breast cancer.

According to our previous study, rs2735385, rs6999227, rs1061302, rs2299941, rs16945628, and rs1805812 are associated with risks of breast cancer under the codominant model in unselected cases involved in the monoubiquitinated FANCD2–DNA damage repair pathway among a chi-square test in 734 Chinese women with breast cancer and 672 age-matched healthy controls. rs1061302 is also associated with susceptibility to lung and upper aerodigestive tract (UADT) cancers (Yang et al., 2014) and the risk of the systemic lupus erythematosus in Taiwanese patients (Lin et al., 2010). rs2735343 is associated with the progression of esophageal squamous carcinoma. But regretfully, we did not find the association between these SNPs and breast cancer, neither rs192236678, rs146605798, rs72550742, rs182030463, rs147494981, rs182756889, rs2735385, rs6999227, rs1805812, rs1061302, rs1042522, rs2735343, rs2299941, and rs16945628 (Table 2). It may be caused by the sample size and the sample type.

There are studies about rs1042522 of gene *TP53*. According to these studies, rs1042522 of gene *TP53* is strongly relevant to tumors between patients and healthy controls (Afzaljavan et al., 2020). The G and C of this polymorphism allele encode an Arg and Pro at position 72 of the P53,

TABLE 3 The logistic analysis of rs7220719

Mutation[a]	B	SEM	Wald	*df*	*p* value	Exp (B)	95% CI	
							Lower limit	Upper limit
Control								
Intercept	50.339	4771.240	0.000	1	0.992			
Size ≤2 cm	−17.418	0.926	353.804	1	0.000	2.725E-8	4.438E-9	1.673E-7
Size 2–5 cm	−1.557	0.952	2.674	1	0.102	.211	0.033	1.363
Size >5 cm	0.458	1.287	0.127	1	0.722	1.581	0.127	19.691
Unknown	0[b]	—	—	0	—	—	—	—
With lymph node	0.233	0.595	0.153	1	0.695	1.262	0.393	4.050
Without lymph node	0[b]	—	—	0	—	—	—	—
Carcinoma in situ	0.489	2.059	0.056	1	0.812	1.630	0.029	92.217
Invasive nonspecific cancer	−.264	1.718	0.024	1	0.878	0.768	0.026	22.269
Invasive specific cancer	−.619	2.296	0.073	1	0.788	0.539	0.006	48.491
Other	0[b]	—	—	0	—	—	—	—
Lymph metastasis = 0	−16.196	2565.176	0.000	1	0.995	9.253E-8	0.000	—[c]
Lymph metastasis 1–3	−16.677	2565.176	0.000	1	0.995	5.718E-8	0.000	—[c]
Lymph metastasis >3	−17.317	2565.176	0.000	1	0.995	3.015E-8	0.000	—[c]
Lymph metastasis unknown	0[b]	—	—	0	—	—	—	—
T = 1	15.909	0.000	—	1	—	8112426.669	8112426.669	8112426.669
T = 2	0[b]	—	—	0	—	—	—	—
T = 3	0[b]	—	—	0	—	—	—	—
T = 4	0[b]	—	—	0	—	—	—	—
N = 0	.308	0.000	—	1	—	1.361	1.361	1.361
N = 1	0[b]	—	—	0	—	—	—	—
M = 0	−.087	1.378	0.004	1	0.950	0.917	0.062	13.650
M = 1	0[b]	—	—	0	—	—	—	—
ER negative	14.827	1988.409	0.000	1	0.994	2750615.587	0.000	—[c]
ER positive	0[b]	—	—	0	—	—	—	—
PR negative	−.528	1816.860	0.000	1	1.000	0.590	0.000	—[c]
PR positive	0[b]	—	—	0	—	—	—	—
HER2 negative	−15.077	1962.413	0.000	1	0.994	2.831E-7	0.000	—[c]
HER2 positive	−15.160	1962.413	0.000	1	0.994	2.608E-7	0.000	—[c]
HER2 unknown	0[b]	—	—	0	—	—	—	—
ki67 ≤30%	−16.054	3633.578	0.000	1	0.996	1.066E-7	0.000	—[c]
ki67 >30%	−15.909	3633.578	0.000	1	0.997	1.233E-7	0.000	—[c]
ki67 unknown	0[b]	—	—	0	—	—	—	—
luminalA	−1.308	1816.860	0.000	1	0.999	0.270	0.000	—[c]
luminalB	−.837	1816.860	0.000	1	1.000	0.433	0.000	—[c]
HER2	−15.502	1988.410	0.000	1	0.994	1.851E-7	0.000	—[c]
TNBC	−15.061	1988.410	.000	1	0.994	2.879E-7	0.000	—[c]
Other	0[b]	—	—	0	—	—	—	—

Abbreviations: ER, estrogen receptor; HER2, ER-, PR-, HER2+; Ki67, antigen identified by monoclonal antibody ki67, a protein which in humans is encoded by the MKI67 gene; luminalA, ER+, PR+, HER2-, ki67<30%; luminalB, ER+, PR+, HER2-, KI67>30%; PR, progesterone receptor; SEM, standard error of mean; TNBC, triple-negative breast cancer, ER-, PR-, HER2-.

[a]^1.

[b]Set to zero.

[c]Floating point overflow, set to system missing values.

TABLE 4 The logistic analysis of rs11871753

Mutation[a]	B	SEM	Wald	*df*	*p* value	Exp (B)	95% CI	
							Lower limit	Upper limit
Control								
Intercept	48.435	4611.289	0.000	1	0.992			
Size ≤2 cm	−16.684	0.895	347.625	1	0.000	5.681E-8	9.835E-9	3.282E-7
Size 2–5 cm	−1.388	0.924	2.256	1	0.133	0.250	0.041	1.527
Size >5 cm	−.047	1.183	0.002	1	0.968	0.954	0.094	9.701
Unknown	0[b]	—	—	0	—	—	—	—
With lymph node	1.110	0.594	3.491	1	0.062	3.035	0.947	9.728
Without lymph node	0[b]	—	—	0	—	—	—	—
Carcinoma in situ	1.836	1.915	0.919	1	0.338	6.272	0.147	267.766
Invasive nonspecific cancer	1.516	1.496	1.027	1	0.311	4.555	0.243	85.539
Invasive specific cancer	−1.027	2.155	0.227	1	0.634	0.358	0.005	24.478
Other	0[b]	—	—	0	—	—	—	—
Lymph metastasis=0	−17.182	2427.652	0.000	1	0.994	3.451E-8	0.000	—[c]
Lymph metastasis 1–3	−17.858	2427.652	0.000	1	0.994	1.755E-8	0.000	—[c]
Lymph metastasis>3	−17.979	2427.652	0.000	1	0.994	1.555E-8	0.000	—[c]
Lymph metastasis unknown	0[b]	—	—	0	—	—	—	—
T = 1	15.744	0.000	—	1	—	6881668.767	6881668.767	6881668.767
T = 2	0[b]	—	—	0	—	—	—	—
T = 3	0[b]	—	—	0	—	—	—	—
T unknown	0[b]	—	—	0	—	—	—	—
N = 0	−.988	0.000	—	1	—	0.372	0.372	0.372
N = 1	0[b]	—	—	0	—	—	—	—
M = 0	−.371	1.577	0.055	1	0.814	0.690	0.031	15.182
M = 1	0[b]	—	—	0	—	—	—	—
ER negative	29.045	1976.039	0.000	1	0.988	4110700766525.211	0.000	—[c]
ER positive	0[b]	—	—	0	—	—	—	—
PR negative	−15.403	1386.464	0.000	1	0.991	2.045E-7	0.000	—[c]
PR positive	0[b]	—	—	0	—	—	—	—
HER2 negative	.857	2.032	0.178	1	0.673	2.356	0.044	126.326
HER2 positive	1.563	2.196	0.507	1	0.477	4.773	0.065	352.898
HER2 unknown	0[b]	—	—	0	—	—	—	—
ki67 ≤30%	−15.680	3667.180	0.000	1	0.997	1.549E-7	0.000	—[c]
ki67 >30%	−15.744	3667.180	0.000	1	0.997	1.453E-7	0.000	—[c]
ki67 unknown	0[b]	—	—	0	—	—	—	—
luminalA	−15.960	1386.464	0.000	1	0.991	1.171E-7	0.000	—[c]
luminalB	−15.832	1386.464	0.000	1	0.991	1.331E-7	0.000	—[c]
HER2	−30.331	1976.040	0.000	1	0.988	6.723E-14	0.000	—[c]
TNBC	−29.095	1976.040	0.000	1	0.988	2.313E-13	0.000	—[c]
Other	0[b]	—	—	0	—	—	—	—

Abbreviations: ER, estrogen receptor; HER2, ER-, PR-, HER2+; Ki67, antigen identified by monoclonal antibody ki67, a protein which in humans is encoded by the MKI67 gene; luminalA, ER+, PR+, HER2-, ki67<30%; luminalB, ER+, PR+, HER2-, KI67>30%; PR, progesterone receptor; SEM, standard error of mean; TNBC: triple-negative breast cancer, ER-, PR-, HER2-.

[a]^1.

[b]Set to zero.

[c]Floating point overflow, set to system missing values.

TABLE 5 The chi-square test of rs7220719

	Classification	Quantity	*p* value
Size	≤2 cm	40	0.144
	2–5 cm	49	
	>5 cm	13	
	Unknown	15	
Lymph node	Without	82	0.402
	With	35	
Histological classification	Carcinoma in situ	6	0.859
	Invasive nonspecific cancer	104	
	Invasive specific cancer	4	
	Other	3	
Lymph metastasis	0	74	0.286
	1–3	26	
	>3	15	
	Unknown	2	
T	1	41	0.153
	2	48	
	3	13	
	Unknown	15	
N	0	81	0.487
	1	36	
M	0	112	0.568
	1	5	
ER	Negative	47	0.450
	Positive	70	
PR	Negative	63	0.443
	Positive	54	
HER2	Negative	74	0.364
	Positive	39	
	Unknown	4	
ki67	≤30%	78	0.328
	>30%	38	
	Unknown	1	
Subtype	luminalA	38	0.212
	luminalB	17	
	HER2	16	
	TNBC	26	
	Other	20	

and the changes in this gene are also frequent among breast cancer patients (Anoushirvani et al., 2018). It also works with WRAP53. WRAP53 is a natural antisense transcript that regulates TP53 transcription and the cell cycle. Certain haplotypes in TP53-WRAP53 locus play an important role in breast cancer susceptibility (Pouladi et al., 2019). But the conclusion has not been unified, and further studies and experiments are needed to investigate the mechanism of this locus. It may for the reason that the sample size is not large enough and the crowd selection offset, and the P value of rs1042522 is larger than 0.05. As this SNP did not accord with Hardy–Weinberg equilibrium in our study, we excluded it from 15 SNPs (Table 1).

This study analyzes the genetic susceptibility of breast cancer from the perspective of clinicopathological features, but we have not performed the functional and clinical significance studies of these SNPs. In addition, although one hundred and seventeen core families are a large sample capacity

TABLE 6 The chi-square test of rs11871753

	Classification	Quantity	*p* value
Size	≤2 cm	40	0.660
	2–5 cm	49	
	>5 cm	13	
	Unknown	15	
Lymph node	Without	82	0.329
	With	35	
Histological classification	Carcinoma in situ	6	0.374
	Invasive nonspecific cancer	104	
	Invasive specific cancer	4	
	Other	3	
Lymph metastasis	0	74	0.441
	1–3	26	
	>3	15	
	Unknown	2	
T	1	41	0.672
	2	48	
	3	13	
	Unknown	15	
N	0	81	0.404
	1	36	
M	0	112	0.594
	1	5	
ER	Negative	47	0.550
	Positive	70	
PR	Negative	63	0.578
	Positive	54	
HER2	Negative	74	0.870
	Positive	39	
	Unknown	4	
ki67	≤30%	78	0.595
	>30%	38	
	Unknown	1	
Subtype	luminalA	38	0.285
	luminalB	17	
	HER2	16	
	TNBC	26	
	Other	20	

for TDT, it is not enough for other analysis. More patients are under 45 so that the age deviation may exist. According to our research, FANCD2 pathway plays a role in DNA double-strand break repair and is not significantly associated with tumor's subtype. The main pathway that influences tumor's phenotype is estrogen and progesterone metabolic pathway (Lopez-Garcia et al., 2010). Thus, further studies need to be developed to research these SNPs in depth.

7 | CONCLUSION

In this family-based study of breast cancer, we have found that two SNPs (rs7220719 and rs11871753) of gene *BRIP1* were significantly associated with the genetic susceptibility of breast cancer. For the first time, we study these related SNPs of several genes in breast cancer by the transmission imbalance of the core families (Machado et al., 2017). Larger

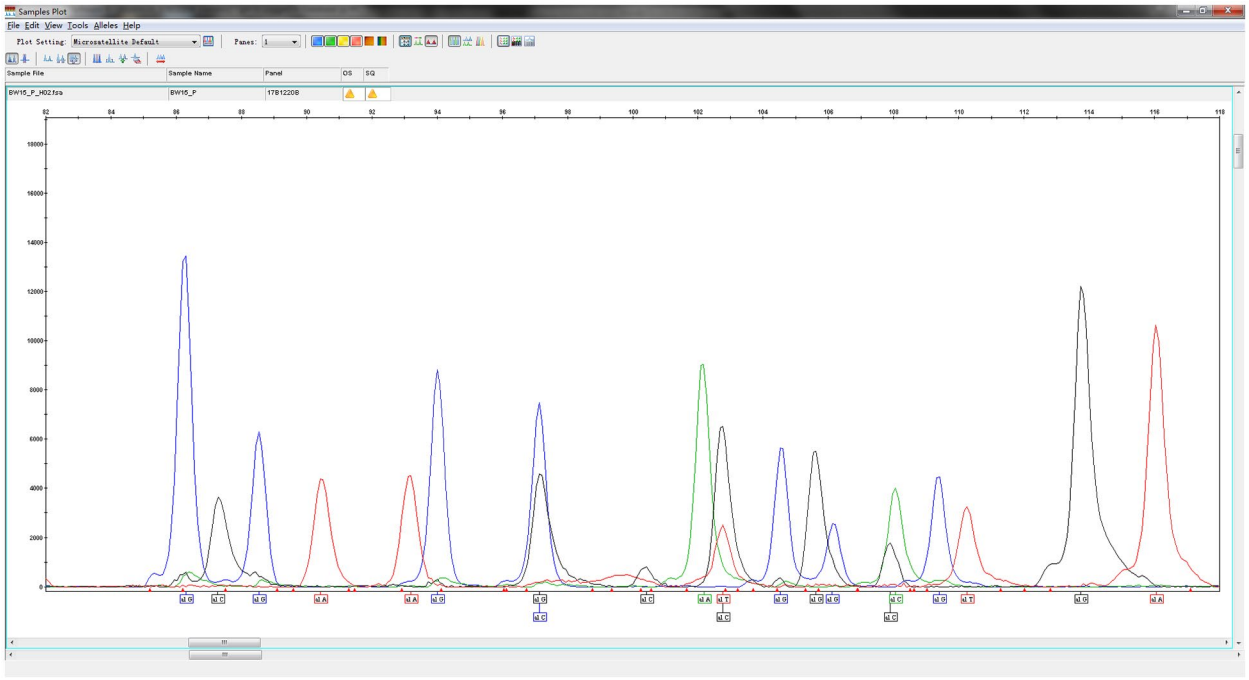

FIGURE 1 The electrophoretogram image of one patient. The orange line stands for the size standard

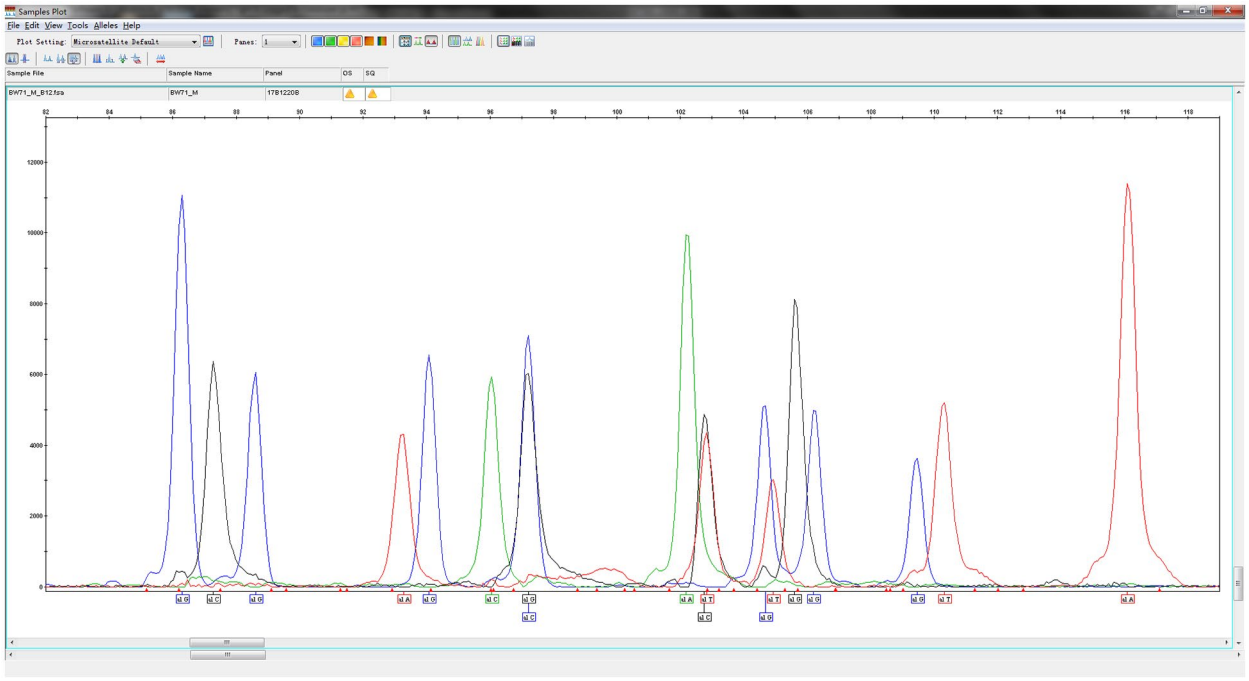

FIGURE 2 The electrophoretogram image of another patient. The orange line stands for the size standard

and deeper studies are needed to confirm their function in breast cancer in the future (Figures 1 and 2).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

The first draft of the manuscript and the data analyzed were written by Xuefei Li. Miao Yang, Yan Luo, and Li Hu collected the patients' information. Zhuo Li and Juan Huang helped to revise the manuscript. Zhi Xiao and Aji Huang helped to design the idea and the project. All the authors read and approved the final manuscript.

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DATA AVAILABILITY STATEMENT

The data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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