Association Between Single-Nucleotide Polymorphisms in Breast Cancer Susceptibility Genes and Clinicopathological Characteristics

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Abstract

Objective The purpose of the present study was to evaluate the associations between seven tagging single nucleotide polymorphisms (tSNPs) and risk of breast cancer assessed by tumor pathological characteristics and body mass index (BMI).

Methods Seven tSNPs of four breast cancer susceptibility genes were analyzed in 734 Chinese women with breast cancer and 672 age-matched healthy controls, then the association with clinicopathological characteristics, BMI, molecular subtype, TNM staging and lymph node status, was determined.

Results Two tSNPs, rs12951053 located in TP53 and rs16945628 in BRIP1, displayed increased risk of breast cancer in the BMI ≧ 25 kg/m^2 group (OR = 1.50, 95% CI: 1.02–2.21, P = 0.041 and OR = 1.92, 95% CI: 1.13–3.26, P = 0.015, respectively). The other five tSNPs (rs1805812, rs2735385 and rs6999227 in NBS1, rs7220719 in BRIP1 and rs2299941 in PTEN) displayed a decreased risk of breast cancer in the 18.5 ≤ BMI < 25 kg/m^2 group. Two tSNPs, rs12951053 in TP53 and rs7220719 in BRIP1 exhibited an increased risk of triple-negative breast cancer (OR = 1.50, 95% CI: 1.05–2.15, P = 0.026 and OR = 2.13, 95% CI: 1.05–4.29, P = 0.032, respectively), and the three tSNPs in NBS1 (rs1805812, rs2735385 and rs6999227) all displayed negative association with both luminal B breast cancer and triple-negative breast cancer. The tSNP rs2299941 in PTEN also exhibited a negative association with each breast cancer molecular subtype, except triple-negative breast cancer. The majority of tSNPs displayed a negative association with stage II or III breast cancer. A number of tSNPs showed a negative association with breast cancer that was lymph node negative or with 1–3 positive nodes. Only one tSNP, rs12951053 in TP53 displayed a positive association with lymph node negative breast cancer (OR = 1.43, 95% CI: 1.08–1.91, P = 0.013).

Conclusions The majority of tSNPs displayed a negative association with breast cancer and only a few tSNPs (rs12951053 in TP53, rs16945628 and rs7220719 in BRIP1) showed an increased risk of breast cancer as defined by clinicopathological characteristics.

Objective

According to the latest statistics, among all malignant tumors in women, the incidence rate of breast cancer ranks highest in both China and the United States[1,2]. The mortality rate ranks second for all female malignant tumors in the United States, with a declining trend year-by-year, attributed to the early detection of breast cancer and individualized treatment according to the molecular classification of the cancer. With the development of breast cancer susceptibility gene panels, evidence that genetic factors can also influence tumor subtype is provided by the fact that patients with triple-negative breast cancer (TNBC) tend to more frequently have mutations in the BRCA1 (8.5%) and BRCA2 (2.7%) genes, in addition to other low-to-medium penetrance genes, including PALB2 (1.2%) and BARD1, RAD51D, RAD51C, and BRIP1 (0.3% to 0.5%)[3]. Furthermore, in patients with HER2-negative metastatic breast cancer and a germline BRCA mutation, monotherapy with the PARP inhibitor olaparib provides a significant benefit over standard therapy, especially in TNBC (OR=0.43, 95% CI: 0.29-0.063)[4]. The majority of previous reports suggest that genetic polymorphisms possibly influence the pathological subtype of breast cancer in only ER-positive or ER-negative breast cancer[5,6,7]. There are only a few published reports of risk factors for combined ER, PR and HER2 status, one of which is a study by O'Brien *et al.* that demonstrates that several SNPs in TNRC9/TOX3 are associated with luminal A or basal-like breast cancer, and one SNP (rs3104746) that is associated with both[8]. Therefore, understanding the associations between genetic polymorphisms and clinicopathological features of breast cancer may ultimately result in improvements in prevention, early detection, and treatment. In a previous study, seven tagging SNPs (tSNPs) of four genes were found to be significantly associated with breast cancer risk under a codominant model in unselected cases, including rs12951053 located in TP53, rs1805812, rs2735385 and rs6999227 in NBS1, rs16945628 and rs7220719 in BRIP1, and rs2299941 in PTEN[9]. Therefore, the purpose of the present study was to evaluate the associations between these tSNPs and breast cancer risk as defined by tumor pathological characteristics and body mass index (BMI).

Materials And Methods

Subjects

As we have described in a previous study[9,10], 734 female patients with pathologically confirmed breast cancer were recruited from the Department of Breast Surgery of Central South University's Xiangya Hospital, Changsha, between January 2007 and October 2011, and the Department of Breast Surgery of the Second People's Hospital of Sichuan Province, Chengdu, China, between November 2010 and May 2011. In addition, a total of 672 aged-matched women with no personal or family history of cancer were enrolled as normal controls at the Health Management Center of Central South University's Xiangya Hospital from July to August 2011. All participants provided signed informed consent prior to blood extraction. The Ethics Committees of Xiangya Hospital of Central South University and the Second People's Hospital of Sichuan Province approved the study.

In a previous study[9], seven tSNPs of four genes were found to be significantly associated with risk of breast cancer when analyzed using a codominant model in unselected cases, including rs12951053 located in TP53, rs1805812, rs2735385 and rs6999227 in NBS1, rs16945628 and rs7220719 in BRIP1, and rs2299941 in PTEN. Finally, in the present study, we analyzed the association between these tSNPs and breast cancer risk using tumor pathological characteristics and body mass index (BMI). SNP genotyping was performed using a custom-by-design 2 × 48-Plex SNPscan kit (cat#: G0104, Genesky Biotechnologies Inc., Shanghai, China), as described in a previous study[9,10]. In total, 728 patients and 671 controls were successfully genotyped for additional analysis.

Clinical Data

BMI was calculated from patient bodyweight and height, BMI = weight (kg)/height squared (m^2). In accordance with the World Health Organization guidelines[11], patients were categorized into three groups: under-weight: BMI < 18.5 kg/m^2; normal weight: 18.5 ≤ BMI < 25 kg/m^2; or overweight: BMI ≧ 25 kg/m^2. Due to a number of missing data points for bodyweight and height, a total of only 496 cases were included in the BMI analysis.

All tissue samples of the patients were analyzed using immunohistochemistry (IHC) by experienced pathologists, including staining of the following markers: ER (estrogen receptor), PR (progesterone receptor), Ki-67 (cell proliferation marker) and HER2 (human epidermal growth factor receptor 2). In accordance with the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines for Breast Cancer, Version 3.2020, breast cancer tumors in which at least 1% of cells stained positive for ER or PR were considered ER- or PR-positive. Tumors were classified as HER2-positive if they scored 3 + using IHC, defined as uniform membrane staining for HER2 in 10% or more of tumor cells, or demonstrating HER2 gene amplification as observed by *in situ* hybridization (ISH). In the present study, if the HER2 marker was scored 2 + by IHC, the sample was further assessed by fluorescence *in situ* hybridization (FISH). As recommended by the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013[12], tumors were grouped into four subtypes depending on the status of four markers (ER, PR, Ki-67 and HER2): Luminal A (ER and PR positive (PR ≥ 20% positive), HER2 negative and Ki-67 low (< 14%)); (2) Luminal B (ER positive, HER2 negative and either: Ki-67 high(≥ 14%) or PR negative or low (< 20% positive); or ER positive, HER2 positive, Ki-67 and PR any status); (3) HER2 positive (HER2 over-expressed or amplified, ER and PR absent); (4) Triple negative (ER and PR absent, HER2 negative). A total of 591 cases were included for subtype analysis, because patients who scored HER2 2 + by IHC without further assessment by FISH were excluded and so some data are missing.

Tumor TNM staging and lymph node status were examined according to the American Joint Committee on Cancer (AJCC) standards in 2010. Due to missing data, a total of 550 cases were investigated for TNM staging analysis and 589 cases for lymph node analysis.

Statistical Methods

Unconditional logistic regression was used to estimate odds ratios (OR) and associated 95% confidence intervals (CI) as measures of association between genotypes and risk of breast cancer subtypes, TNM staging, lymph node status and BMI (comparing case subtypes to common homozygote controls). Statistical analysis was conducted using SPSS v.24.0 software.

Results

Association between tSNPs and Breast Cancer Risk by Body Mass Index

As described in Table 1, in the BMI ≧ 25 kg/m^2 group, compared with the common homozygote, the heterozygote C/A of tSNP rs12951053 in TP53 displayed an increased risk of breast cancer (OR = 1.50, 95% CI: 1.02–2.21, P = 0.041). The same trend also was observed in tSNP rs16945628 in BRIP1, but for the uncommon homozygote T/T (OR = 1.92, 95% CI: 1.13–3.26, P = 0.015). Conversely, the uncommon homozygote G/G of tSNP rs2299941 in PTEN demonstrated a decreased risk of breast cancer (OR = 0.49, 95% CI: 0.24–0.99, P = 0.042). No significant associations were observed in the BMI < 18.5 kg/m^2 group.

Table 1
Odds ratios and 95% confidence intervals for breast cancer risk by body mass index

				BMI(kg/m^2)								
			controls	< 18.5			18.5-24.99			≥ 25		
Gene	tSNP	Genetype	N	N	OR[a](95%CI)	*P*-value[b]	N	OR[a](95%CI)	*P*-value[b]	N	OR[a](95%CI)	*P*-value[b]
TP53	rs12951053	A/A	331	12	1.00		153	1.00		55	1.00	
		C/A	273	11	1.11(0.48–2.56)	0.804	156	1.24(0.94–1.63)	0.130	68	**1.50(1.02–2.21)**	**0.041**
		C/C	67	2	0.82(0.18–3.76)	0.802	23	0.74(0.45–1.24)	0.253	15	1.35(0.72–2.53)	0.351
NBS1	rs 1805812	T/T	470	20	1.00		254	1.00		100	1.00	
		C/T	184	5	0.64(0.24–1.73)	0.373	68	**0.68(0.50–0.94)**	**0.019**	34	0.87(0.57–1.33)	0.515
		C/C	16	0	/	/	11	1.27(0.58–2.78)	0.546	4	1.18(0.39–3.59)	0.777
	rs 2735385	C/C	210	8	1.00		127	1.00		54	1.00	
		C/A	345	14	1.07(0.44–2.58)	0.889	161	0.77(0.58–1.03)	0.078	61	0.69(0.46–1.03)	0.069
		A/A	116	3	0.68(0.18–2.61)	0.571	44	**0.63(0.42–0.95)**	**0.026**	23	0.77(0.45–1.32)	0.343
	rs6999227	G/G	200	7	1.00		124	1.00		49	1.00	
		G/C	344	14	1.16(0.46–2.93)	0.749	159	**0.75(0.56–0.99)**	**0.049**	64	0.76(0.50–1.15)	0.188
		C/C	126	4	0.91(0.26–3.16)	0.878	50	**0.64(0.43–0.95)**	**0.027**	25	0.81(0.48–1.38)	0.436
BRIP1	rs16945628	C/C	271	10	1.00		154	1.00		46	1.00	
		C/T	313	12	1.04(0.44–2.44)	0.930	135	0.76(0.57–1.01)	0.056	64	1.21(0.80–1.82)	0.376
		T/T	86	3	0.95(0.25–3.51)	0.933	43	0.88(0.58–1.33)	0.546	28	**1.92(1.13–3.26)**	**0.015**
	rs7220719	G/G	429	17	1.00		235	1.00		88	1.00	
		G/A	217	6	0.70(0.27–1.80)	0.453	79	**0.67(0.49–0.90)**	**0.008**	41	0.92(0.61–1.38)	0.691
		A/A	25	2	2.02(0.44–9.23)	0.355	18	1.31(0.70–2.50)	0.391	9	1.76(0.79–3.89)	0.161
PTEN	rs2299941	A/A	268	12	1.00		168	1.00		65	1.00	
		G/A	314	11	0.78(0.34–1.80)	0.563	132	**0.67(0.51–0.89)**	**0.005**	63	0.83(0.56–1.21)	0.331
		G/G	85	2	0.53(0.12–2.40)	0.398	32	**0.60(0.38–0.94)**	**0.025**	10	**0.49(0.24–0.99)**	**0.042**

[a]Compared with common homozygote by logistic regression analysis.

[b]P value for every genetype when compared with common homozygote by logistic regression analysis.

In the 18.5 ≤ BMI < 25 kg/m^2 group, five tSNPs displayed a decreased risk of breast cancer compared with the common homozygote, including two heterozygous genotypes (C/T of tSNP rs1805812 in NBS1, OR = 0.68, 95% CI: 0.50–0.94, *P* = 0.019; and G/A of tSNP rs7220719 in BRIP1, OR = 0.67, 95% CI: 0.49–0.90, *P* = 0.008), the uncommon homozygote A/A of tSNP rs2735385 in NBS1 (OR = 0.63, 95% CI: 0.42–0.95, *P* = 0.026), and two tSNPs containing both heterozygous genotypes and uncommon homozygous genotypes (G/C and C/C of tSNP rs6999227 of NBS1, OR = 0.75, 95% CI: 0.56–0.99, *P* = 0.049; OR = 0.64, 95% CI: 0.43–0.95, *P* = 0.027 respectively, G/A and G/G of tSNP rs2299941 of PTEN, OR = 0.67, 95% CI: 0.51–0.89, *P* = 0.005; OR = 0.60, 95% CI: 0.38–0.94, *P* = 0.025 respectively) (Table 1).

Association between tSNPs and Breast Cancer Risk by Tumor Pathological Characteristics

As presented in Table 2, luminal A (44.3%) was the subtype of breast cancer in highest proportion in the present study, followed by triple-negative (27.8%), the lowest being the HER-2 positive/HR negative subtype (5.8%), similar to the proportion in our previous reports[13].

Table 2
The association between the each breast cancer molecular subtype and tSNPs,relative to controls

Gene	tSNP	Gene type	Controls N[a]=671	Luminal A N[a]=261			Luminal B N[a]=130			HER2+/HR- N[a]=34			Tr N
			N	N	OR[b](95%CI)	*P*[c]	N	OR[b](95%CI)	*P*[c]	N	OR[b](95%CI)	*P*[c]	N
TP53	rs12951053	A/A	331	121	1.00		53	1.00		14	1.00		6
		C/A	273	119	1.19(0.88–1.61)	0.249	64	1.46(0.98–2.18)	0.059	18	1.56(0.76–3.19)	0.221	8
		C/C	67	21	0.86(0.50–1.46)	0.571	13	1.21(0.63–2.35)	0.569	2	0.71(0.16–3.18)	0.648	1
		Per allele			1.03(0.82–1.28)	0.828		1.22(0.92–1.21)	0.171		1.10(0.65–1.85)	0.723	
NBS1	rs1805812	T/T	470	187	1.00		106	1.00		27	1.00		1
		C/T	184	67	0.92(0.66–1.27)	0.595	21	**0.51(0.31–0.83)**	**0.007**	6	0.57(0.23–1.40)	0.212	3
		C/C	16	9	1.41(0.61–3.26)	0.414	3	0.83(0.24–2.91)	0.772	1	1.09(0.14–8.51)	0.936	3
		Per allele			1.00(0.76–1.32)	0.983		**0.60(0.40–0.92)**	**0.018**		0.69(0.33–1.47)	0.338	
	rs2735385	C/C	210	97	1.00		57	1.00		13	1.00		7
		C/A	345	126	0.79(0.58–1.08)	0.144	59	**0.63(0.42–0.94)**	**0.024**	19	0.89(0.43–1.84)	0.752	7
		A/A	116	40	0.75(0.48–1.15)	0.185	14	**0.45(0.24–0.83)**	**0.010**	2	0.28(0.06–1.26)	0.077	1
		Per allele			0.85(0.70–1.05)	0.131		**0.67(0.50–0.88)**	**0.004**		0.68(0.41–1.13)	0.136	
	rs6999227	G/G	200	92	1.00		56	1.00		12	1.00		6
		G/C	344	129	0.82(0.59–1.12)	0.209	56	**0.58(0.39–0.88)**	**0.009**	20	0.97(0.46–2.02)	0.933	7
		C/C	126	42	0.73(0.47–1.11)	0.139	18	**0.51(0.29–0.91)**	**0.020**	2	0.27(0.06–1.20)	0.065	2
		Per allele			0.85(0.69–1.04)	0.118		**0.68(0.52–0.90)**	**0.007**		0.68(0.41–1.13)	0.137	
BRIP1	rs16945628	C/C	271	106	1.00		55	1.00		18	1.00		7
		C/T	313	115	0.94(0.69–1.28)	0.692	52	0.82(0.54–1.24)	0.341	12	0.58(0.27–1.22)	0.146	6
		T/T	86	41	1.22(0.79–1.88)	0.372	23	1.32(0.77–2.27)	0.319	4	0.70(0.23–2.13)	0.53	2
		Per allele			1.06(0.86–1.31)	0.572		1.07(0.81–1.40)	0.646		0.74(0.43–1.25)	0.256	
	rs7220719	G/G	429	182	1.00		88	1.00		22	1.00		1
		G/A	217	64	**0.70(0.50–0.97)**	**0.030**	37	0.83(0.55–1.26)	0.385	12	1.08(0.52–2.22)	0.838	4
		A/A	25	17	1.60(0.85–3.04)	0.145	5	0.98(0.36–2.62)	0.960	0			1
		Per allele			0.92(0.71–1.19)	0.536		0.89(0.63–1.25)	0.499		0.86(0.46–1.63)	0.65	
PTEN	rs2299941	A/A	268	128	1.00		61	1.00		22	1.00		8
		G/A	314	109	**0.73(0.54–0.98)**	**0.039**	60	0.84(0.57–1.24)	0.381	11	**0.43(0.20–0.90)**	**0.021**	6

[a]Number for all Controls and patients with diferent breast cancer molecular subtype,but some of them were unsuccessfully genetyped in some tSNPs.

[b]Compared with common homozygote and common per allele by logistic regression analysis.

[c]P value for every genetype when compared with common homozygote,and per allete when compared with common per allele by logistic regression analysis

Gene	tSNP	Gene type	Controls N[a]=671	Luminal A N[a]=261			Luminal B N[a]=130			HER2+/HR- N[a]=34			T N
		G/G	85	26	0.64(0.39–1.04)	0.072	8	**0.41(0.19–0.90)**	**0.022**	1	0.14(0.02–1.08)	0.029	1
		Per allele			**0.78(0.62–0.96)**	**0.021**		**0.73(0.55–0.98)**	**0.036**		**0.42(0.22–0.77)**	**0.004**	

[a]Number for all Controls and patients with diferent breast cancer molecular subtype,but some of them were unsuccessfully genetyped in some tSNPs.

[b]Compared with common homozygote and common per allele by logistic regression analysis.

[c]P value for every genetype when compared with common homozygote,and per allete when compared with common per allele by logistic regression analysis

Four tSNPs were negatively associated with luminal B breast cancer compared with the common homozygote (Table 2). Of these, two tSNPs in NBS1 (tSNP rs2735385 and rs6999227) contained both heterozygous genotypes and uncommon homozygous genotypes that displayed a negative association, OR = 0.63, 95% CI: 0.42–0.94, *P* = 0.024 and OR = 0.45, 95% CI: 0.24–0.83, *P* = 0.010 for the C/A and A/A genotypes of rs2735385, respectively; OR = 0.58, 95% CI: 0.39–0.88, *P* = 0.009 and OR = 0.51, 95% CI: 0.29–0.91, *P* = 0.020 for the G/C and C/C genotypes of rs6999227, respectively (Table 2). The tSNP rs1805812 in NBS1 contained only a heterozygous C/T genotype that exhibited a negative association (OR = 0.51, 95% CI: 0.31–0.83, *P* = 0.007). The tSNP rs2299941 in PTEN contained only the uncommon homozygous genotype G/G that displayed a negative association (OR = 0.41, 95% CI: 0.19–0.90, *P* = 0.022). Each single allele of the four tSNPs also exhibited a negative association (OR = 0.60–0.73) (Table 2).

Several tSNPs were strongly associated with triple-negative breast cancer (Table 2). Patients with the C/A heterozygote tSNP rs12951053 located in TP53 exhibited a risk of triple-negative breast cancer 1.50-fold greater (95% CI: 1.05–2.15, *P* = 0.026) than those with the common homozygote A/A. The uncommon homozygous genotype A/A of tSNP rs7220719 in BRIP1 displayed a higher risk than that described above (OR = 2.13, 95% CI: 1.05–4.29, *P* = 0.032). The tSNPs, rs1805812 and rs2735385 of NBS1, displayed a negative association with triple-negative breast cancer compared with the common homozygote (OR = 0.61, 95% CI: 0.40–0.93, *P* = 0.022 for genotype C/T of rs1805812; OR = 0.64, 95% CI: 0.45–0.93, *P* = 0.019 for genotype C/A and OR = 0.49, 95% CI: 0.28–0.86, *P* = 0.011 for genotype A/A of rs2735385). Each single allele of the two tSNPs also exhibited a negative association (OR = 0.66, 95% CI: 0.46–0.96, *P* = 0.029; OR = 0.69, 95% CI: 0.54–0.89, *P* = 0.004, respectively). Only one allele of two other tSNPs showed a negative association with triple-negative breast cancer (OR = 0.77, 95% CI: 0.60–0.99, *P* = 0.037 for rs6999227 in NBS1 and OR = 0.74, 95% CI: 0.57–0.97, *P* = 0.027 for rs2299941 in PTEN) (Table 2).

Only two tSNPs with a heterozygous genotype exhibited a negative association with luminal A breast cancer, OR = 0.70, 95% CI: 0.50–0.97, *P* = 0.030 for genotype G/A of rs7220719 in BRIP1 and OR = 0.73, 95% CI :0.54–0.98, *P* = 0.039 for genotype G/A of rs2299941 in PTEN (Table 2). The same trend was observed for each single allele of rs2299941 (OR = 0.78, 95% CI: 0.62–0.96, *P* = 0.021). The heterzygote G/A of rs2299941 in PTEN also exhibited a negative association with HER-2 positive/HR negative breast cancer (OR = 0.43, 95% CI: 0.20–0.90, *P* = 0.021), each single allele displaying the same trend (OR = 0.42, 95% CI: 0.22–0.77,*P* = 0.004) (Table 2).

As displayed in Table 3, three tSNPs (rs2735385 and rs6999227 in NBS1, rs2299941 in PTEN) in both heterozygous genotypes and the uncommon homozygous genotypes, displayed a negative association with stage II breast cancer compared with the common homozygote (OR = 0.71, 95% CI: 0.53–0.95, *P* = 0.021 and OR = 0.58, 95% CI: 0.38–0.89, *P* = 0.011 for genotypes C/A and A/A of rs2735385, respectively; OR = 0.70, 95% CI: 0.52–0.94, *P* = 0.018 and OR = 0.61, 95% CI: 0.41–0.92, *P* = 0.016 for genotypes G/C and C/C of rs6999227, respectively; OR = 0.73, 95% CI: 0.55–0.97,*P* = 0.028 and OR = 0.48, 95% CI: 0.29–0.79, *P* = 0.003 for genotypes G/A and G/G of rs2299941, respectively). The two other tSNPs with only heterozygous genotypes also displayed a negative association with stage II breast cancer (OR = 0.70, 95% CI: 0.50–0.96, *P* = 0.027 for genotype C/T of rs1805812 in NBS1 and OR = 0.68, 95% CI: 0.50–0.93, *P* = 0.015 for genotype G/A of rs7220719 in BRIP1). Only one heterozygote, the genotype C/A of tSNP rs12951053 located in TP53 exhibited a positive association with stage II breast cancer (OR = 1.54, 95% CI: 1.16–2.04, *P* = 0.003).

Table 3
Oddi ratios and 95% confidence intervals for the association between tSNPs and each TNM staging

				TNM staging								
			controls	Stage I			Stage II			Stage III		
Gene	tSNP	Genetype	N	N	OR[a](95%CI)	*P*-value[b]	N	OR[a](95%CI)	*P*-value[b]	N	OR[a](95%CI)	*P*-value[b]
TP53	rs12951053	A/A	331	28	1.00		126	1.00		83	1.00	
		C/A	273	30	1.30(0.76–2.23)	0.341	160	**1.54(1.16–2.04)**	**0.003**	76	1.11(0.78–1.58)	0.558
		C/C	67	3	0.53(0.16–1.79)	0.299	28	1.10(0.68–1.79)	0.707	14	0.83(0.45–1.56)	0.567
NBS1	rs 1805812	T/T	470	46	1.00		239	1.00		133	1.00	
		C/T	184	14	0.78(0.42–1.45)	0.427	65	**0.70(0.50–0.96)**	**0.027**	38	0.73(0.49–1.09)	0.121
		C/C	16	1	0.64(0.08–4.93)	0.664	12	1.48(0.69–3.17)	0.316	2	0.44(0.10–1.95)	0.267
	rs 2735385	C/C	210	26	1.00		127	1.00		75	1.00	
		C/A	345	27	0.63(0.36–1.11)	0.109	148	**0.71(0.53–0.95)**	**0.021**	75	**0.61(0.42–0.88)**	**0.007**
		A/A	116	8	0.56(0.24–1.27)	0.159	41	**0.58(0.38–0.89)**	**0.011**	22	**0.53(0.31–0.90)**	**0.017**
	rs6999227	G/G	200	24	1.00		122	1.00		68	1.00	
		G/C	344	28	0.68(0.38–1.20)	0.182	147	**0.70(0.52–0.94)**	**0.018**	85	0.73(0.51–1.05)	0.085
		C/C	126	9	0.60(0.27–1.32)	0.199	47	**0.61(0.41–0.92)**	**0.016**	20	**0.47(0.27–0.81)**	**0.006**
BRIP1	rs16945628	C/C	271	26	1.00		139	1.00		65	1.00	
		C/T	313	29	0.97(0.56–1.68)	0.902	132	0.82(0.62–1.10)	0.183	75	1.00(0.69–1.45)	0.996
		T/T	86	6	0.73(0.29–1.83)	0.496	43	0.98(0.64–1.48)	0.905	33	1.60(0.99–2.60)	0.056
	rs7220719	G/G	429	38	1.00		220	1.00		104	1.00	
		G/A	217	18	0.94(0.52–1.68)	0.826	76	**0.68(0.50–0.93)**	**0.015**	59	1.12(0.78–1.61)	0.531
		A/A	25	5	2.26(0.82–6.24)	0.107	19	1.48(0.80–2.75)	0.21	10	1.65(0.77–3.54)	0.195
PTEN	rs2299941	A/A	268	23	1.00		157	1.00		90	1.00	
		G/A	314	33	1.23(0.70–2.14)	0.475	134	**0.73(0.55–0.97)**	**0.028**	65	**0.62(0.43–0.88)**	**0.008**
		G/G	85	5	0.69(0.25–1.86)	0.456	24	**0.48(0.29–0.79)**	**0.003**	18	0.63(0.36–1.11)	0.106

[a]Compared with common homozygote by logistic regression analysis.

[b]P value for every genetype when compared with common homozygote by logistic regression analysis.

In stage III breast cancer, only three tSNPs were found to have a negative association, a heterozygous genotype and an uncommon homozygote genotype (OR = 0.61, 95% CI: 0.42–0.88, *P* = 0.007 and OR = 0.53, 95% CI: 0.31–0.90, *P* = 0.017 for genotypes C/A and A/A of rs2735385 in NBS1, respectively), the other a heterozygous genotype (OR = 0.62, 95% CI:0.43–0.88, *P* = 0.008 for genotype G/A of rs2299941 inof PTEN) and another, an uncommon homozygote (OR = 0.47, 95% CI: 0.27–0.81, *P* = 0.006 for genotype C/C of rs6999227 in NBS1) (Table 3). No significant associations with stage I breast cancer were identified.

Analysis of the association between tSNPs and lymph node status of breast cancer indicated that a number of tSNPs displayed a negative association with lymph node negative breast cancer (rs1805812, rs2735385 and rs6999227 in NBS1, rs7220719 in BRIP1 and rs2299941 in PTEN, OR = 0.51–0.73) (Table 4). Only one heterozygous genotype, C/A of tSNP rs12951053 in TP53 exhibited a positive association with lymph node negative breast cancer (OR = 1.43, 95% CI: 1.08–1.91, *P* = 0.013). We also found three tSNPs (rs2735385 and rs6999227 in NBS1 and rs2299941 in PTEN, OR = 0.53–0.67) that were negatively associated with breast cancers having 1–3 positive nodes (Table 4). The same trend was observed in breast cancer with more than three positive nodes (rs1805812 and rs2735385 in NBS1 and rs2299941 in PTEN, OR = 0.53–0.61) (Table 4).

Table 4
Oddi ratios and 95% confidence intervals for the association between tSNPs and lymph node status

				Number of positive nodes								
			controls	0			1–3			>3		
Gene	tSNP	Genetype	N	N	OR[a](95%CI)	*P*-value[b]	N	OR[a](95%CI)	*P*-value[b]	N	OR[a](95%CI)	*P*-value[b]
TP53	rs12951053	A/A	331	126	1.00		75	1.00		54	1.00	
		C/A	273	149	**1.43(1.08–1.91)**	**0.013**	77	1.25(0.87–1.78)	0.228	56	1.26(0.84–1.89)	0.269
		C/C	67	28	1.10(0.68–1.79)	0.707	13	0.86(0.45–1.63)	0.637	9	0.82(0.39–1.75)	0.612
NBS1	rs 1805812	T/T	470	236	1.00		119	1.00		93	1.00	
		C/T	184	58	**0.63(0.45–0.88)**	**0.006**	45	0.97(0.66–1.42)	0.859	21	**0.58(0.35–0.95)**	**0.030**
		C/C	16	10	1.25(0.56–2.79)	0.594	2	0.49(0.11–2.18)	0.342	5	1.58(0.57–4.42)	0.380
	rs 2735385	C/C	210	121	1.00		69	1.00		51	1.00	
		C/A	345	140	**0.70(0.52–0.95)**	**0.021**	76	**0.67(0.46–0.97)**	**0.033**	51	**0.61(0.40–0.93)**	**0.021**
		A/A	116	43	**0.64(0.43–0.98)**	**0.037**	21	**0.55(0.32–0.94)**	**0.029**	16	0.57(0.31–1.04)	0.065
	rs6999227	G/G	200	115	1.00		64	1.00		45	1.00	
		G/C	344	138	**0.70(0.52–0.94)**	**0.020**	83	0.75(0.52–1.09)	0.134	57	0.74(0.48–1.13)	0.160
		C/C	126	51	0.70(0.47–1.05)	0.083	19	**0.47(0.27–0.82)**	**0.007**	17	0.60(0.33–1.09)	0.093
BRIP1	rs16945628	C/C	271	138	1.00		64	1.00		45	1.00	
		C/T	313	123	0.77(0.58–1.03)	0.082	76	1.03(0.71–1.49)	0.883	53	1.02(0.66–1.57)	0.929
		T/T	86	41	0.94(0.61–1.43)	0.761	26	1.28(0.76–2.15)	0.348	21	1.47(0.83–2.61)	0.185
	rs7220719	G/G	429	207	1.00		107	1.00		78	1.00	
		G/A	217	76	**0.73(0.53–0.99)**	**0.042**	52	0.96(0.66–1.39)	0.832	33	0.84(0.54–1.30)	0.424
		A/A	25	20	1.66(0.90–3.05)	0.102	7	1.12(0.47–2.67)	0.793	8	1.76(0.77–4.04)	0.178
PTEN	rs2299941	A/A	268	136	1.00		90	1.00		66	1.00	
		G/A	314	145	0.91(0.68–1.21)	0.517	61	**0.58(0.40–0.83)**	**0.003**	41	**0.53(0.35–0.81)**	**0.003**
		G/G	85	22	**0.51(0.31–0.85)**	**0.009**	15	**0.53(0.29–0.96)**	**0.033**	12	0.57(0.30–1.11)	0.096

[a]Compared with common homozygote by logistic regression analysis.

[b]P value for every genetype when compared with common homozygote by logistic regression analysis.

Discussion

With the development of breast cancer susceptibility gene panels, previous studies have demonstrated that genetic factors can also influence tumor subtype, finding that in TNBC metastatic patients with a germline BRCA mutation, monotherapy with the PARP inhibitor olaparib provided a significant benefit over standard therapy[3,4]. Thus, understanding the associations between genetic polymorphisms and clinicopathological features of breast cancer may ultimately result in improvements in prevention, early detection, and treatment. In a previous study, we found that seven tagging SNPs(tSNPs) of four genes were significantly associated with breast cancer risk using a codominant model in unselected cases[9]. In the present study, we evaluated the associations between these tSNPs and breast cancer risk defined by tumor pathological characteristics and BMI.

Compared with the common homozygote, we found that the heterozygote C/A of tSNP rs12951053 located in TP53 and the uncommon homozygote T/T of tSNP rs16945628 located in BRIP1 displayed an increased risk of breast cancer in the BMI ≧ 25 kg/m^2 group. Conversely, in the 18.5 ≤ BMI < 25 kg/m^2 group,

five tSNPs exhibited a decreased risk of breast cancer compared with the common homozygote, including heterozygous genotypes and uncommon homozygotes (C/T of tSNP rs1805812, A/A of tSNP rs2735385, G/C and C/C of tSNP rs6999227 located in NBS1 and G/A of tSNP rs7220719 located in BRIP1). In addition, the uncommon homozygote G/G of tSNP rs2299941 located in PTEN displayed a decreased risk of breast cancer both in the 18.5 ≤ BMI < 25 kg/m^2 and BMI ≧ 25 kg/m^2 groups. However, no significant associations were found in the BMI < 18.5 kg/m^2 group. With a trend the same as tSNP rs12951053, one study has shown that SNP rs1042522 in TP53 may be associated with susceptibility to breast cancer among Iranian women with high BMI[14].

In TP53, compared with the common homozygote A/A of tSNP rs12951053, patients with the heterozygote C/A had a risk 1.50-fold higher than triple-negative breast cancer, and displayed a positive association with stage II breast cancer and lymph node negative breast cancer. However, a study of Jordanian women did not find a significant association between SNP rs12951053 and breast cancer subtype, tumor stage or lymph node status[15].

In the present study, three tSNPs in NBS1 (rs1805812, rs2735385 and rs6999227 with a heterozygous genotype, uncommon homozygous genotype or individual allele) showed in each case a negative association with luminal B and triple-negative breast cancer, compared with the common homozygote. However, we did not find any significant association with luminal A and HER2+/HR- breast cancer. In stage II breast cancer, we also found that the three tSNPs displayed a negative association compared with the common homozygote. The tSNPa rs2735385 and rs6999227 also exhibited a negative association with stage III breast cancer. The majority of the three tSNPs displayed a negative association with lymph node status.

In BRIP1, we found that the heterozygous genotype G/A of tSNP rs7220719 displayed a negative association with luminal A breast cancer and the uncommon homozygote A/A exhibited a positive association with triple-negative breast cancer. However, we did not find any significant association between tSNP rs16945628 and breast cancer molecular subtype, TNM staging or lymph node status. In addition, heterozygous genotype G/A of tSNP rs7220719 also displayed a negative association with stage II breast cancer and lymph node negative breast cancer.

In tSNP rs2299941 in PTEN, we found that the single allele G displayed a negative association with each breast cancer molecular subtype, in comparison with the A allele. The heterozygous genotype G/A or uncommon homozygous genotype G/G also displayed a negative association with each breast cancer molecular subtype, except triple-negative breast cancer, in comparison with the common homozygote A/A. The same trend was observed with lymph node status. We also found that the heterozygote genotype G/A or uncommon homozygous genotype G/G displayed a negative association with stage II or III breast cancer.

The four genes detailed above are directly or indirectly involved in the monoubiquitinated FANCD2–DNA damage repair pathway and have been found to be significantly associated with risk of breast cancer[16]. A previous study of Chinese patients with familial breast/ovarian cancer found that 135 patients were found to carry pathogenic or likely pathogenic mutations (28.1%), corresponding to 12 different cancer predisposition genes (8.5% on non-BRCA1/2 genes, mainly in ATM, CHEK2, PALB2, NBS1 and BRIP1 genes, all of which participate in the homologous recombinant repair pathway). Mutation rates of BRCA1 and BRCA2 have been shown to be higher in triple-negative breast cancer (TNBC) patients than in the non-TNBC group[17]. The majority of previous reports suggest that genetic polymorphisms may influence the pathological subtype of breast cancer only in ER-positive or -negative breast cancer. Only a few studies have investigated risk factors for combined ER, PR, HER2 status, for example a study by O'Brien *et al*. that demonstrated that a number of SNPs in TNRC9/TOX3 are associated with luminal A or basal-like breast cancer, with one SNP (rs3104746) associated with both. Therefore, it is difficult to compare the results of the present study with previous research.

Conclusions

In this hospital-based case-control study of associations between seven tSNPs and risk of breast cancer defined by tumor pathological characteristics and BMI, we found that the majority of tSNPs displayed a negative association with breast cancer and only a few tSNPs exhibited an increased risk of breast cancer (such as rs12951053 in TP53 and rs7220719 in BRIP1, which displayed an increased risk of triple-negative breast cancer, and rs12951053 of TP53 and rs16945628 of BRIP1, which displayed an increased risk of breast cancer in the BMI ≧ 25 kg/m^2 group). Three tSNPs located in NBS1 (rs1805812, rs2735385 and rs6999227) displayed a negative association with luminal B and triple-negative breast cancer. The tSNP rs2299941 located in PTEN also showed a negative association with each breast cancer molecular subtype except triple-negative breast cancer. The majority of tSNPs displayed a negative association with stage II or III breast cancer. Several tSNPs exhibited a negative association with breast cancer that was lymph node negative or with 1–3 positive nodes. Only one tSNP, rs12951053 located in TP53 showed a positive association with lymph node negative breast cancer. However, the small sample size of the study represents a limitation. Larger and multicenter national studies are required to verify these findings and further exploration of the function of these genes is also required.

Declarations

Ethics approval and consent to participate

The Ethics Committees of Xiangya Hospital of Central South University and the Second People's Hospital of Sichuan Province approved the study.

Consent for publication

Not applicable

Availability of data and materials

The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

Each author is expected to have made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; or have drafted the work or substantively revised it and to have approved the submitted version (and any substantially modified version that involves the author's contribution to the study);and to have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

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