LRRC3B polymorphisms contributed to breast cancer susceptibility in Chinese Han Population

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Abstract

Purpose

LRRC3B gene, as a tumor suppressor gene was involved in the development and progress of breast cancer (BC). However, the effect of *LRRC3B* polymorphisms on BC has rarely been reported. In the study, we aims to evaluate the relation between *LRRC3B* variants and BC risk.

Methods

Among 563 BC patients and 552 healthy controls, ten single-nucleotide polymorphisms (SNPs) in *LRRC3B* were genotyped by Agena MassARRAY. Odds ratios (OR) and 95% confidence interval (CI) was calculate using logistic regression model.

Results

Our study demonstrated that rs1907168 polymorphism (OR = 0.71, $p = 0.017$) reduced the risk of BC in the overall. In stratified analyses by age, rs1907168 decreased (OR = 0.53, $p = 0.002$) while rs78205284 (OR = 2.83, $p = 0.034$) increased BC susceptibility among the population at age ≤ 51 years. Clinical parameters such as tumor size, the status of PR and Ki67 were associated with *LRRC3B* variants. Furthermore, we found that the association of 'GATT' haplotype with an increased risk for BC. In addition, *LRRC3B* gene was down-regulated in BC tumor and had a poor prognosis in BC *in silico* analysis.

Conclusion

Our study firstly found *LRRC3B* SNPs contributed to the risk of BC, suggesting *LRRC3B* variants might help to predict BC progression.

Introduction

Breast cancer (BC) is the most commonly diagnosed malignant tumor and the primary cancer death among women globally [1]. In China, 268,600 new cases of BC were diagnosed and there were 69,500 deaths, and its morbidity and mortality rates have been increasing annually [2]. BC is a multi-factorial, polygenic disease resulting from genetic, endocrine and environmental factors [3]. Age, serum hormone levels, family history and environmental factors have been identified as potential contributors BC susceptibility [4]. Moreover, genetic factors were the major drivers in the genesis of BC [5–7].

Leucine-rich repeat-containing 3B (LRRC3B), also named LRP15, is one of the transmembrane protein, having an evolutionarily conserved LRR-domain [8]. LRR-containing proteins participated in protein interaction, cell adherence, DNA repair, gene recombination and so on[9]. LRRC3B protein has reported to regulate the DNA damage and repair pathways and cell cycle progression [10]. Additionally, *LRRC3B* is down-regulated in in non-small-cell lung, head and neck, gastric and breast cancers [11–14], suggesting *LRRC3B* involvement in carcinogenesis. Abnormal expression of *LRRC3B* in BC was associated with the development and prognosis of BC [13]. In addition, *LRRC3B* also served an important role in BC recurrence and metastasis[15]. Genetic polymorphisms may affect its gene expression, thus associated with the occurrence of disease. In spite of many researchers reported the role of *LRRC3B* expression in BC pathogenesis, the possible effect of *LRRC3B* polymorphisms on BC have not been studied.

Here, we investigated the frequencies distribution of *LRRC3B* polymorphisms and the potential relationship of *LRRC3B* polymorphisms with BC susceptibility in the Chinese Han females.

Materials And Methods

Study subjects

1115 unrelated Chinese Han female subjects (563 BC patients and 552 healthy controls) were enrolled from the Shaanxi Provincial Cancer Hospital. BC patients had newly diagnosed and were confirmed by histopathology based on the WHO BC classification. The blood samples of patients was collected before chemotherapy, radiotherapy, or endocrine therapy. Patients had no any other cancer or other systemic inflammatory disease. At the mean time, age-matched healthy individuals who had a checkup at the hospital were recruited as controls. The controls had no mammary tumors by recent clinical and mammographic examinations, no cancer history, no BC family history and no mammary diseases. The study was approved by the Ethics Committee of Xizang Minzu University and in accordance with the 1964 Helsinki Declaration. Written informed consent was obtained.

Data collection and DNA extraction

Questionnaires and medical records were used to gather the demographic and clinical features. The following data were recorded: age, estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and Ki-67 status, clinical stage, tumor size and tumor lymph node invasion. After interview, peripheral blood (5 mL) was gathered into EDTA-coated tubes from patients and healthy controls. Genomic DNA was isolated using the GoldMag genomic DNA purification kit (GoldMag Co. Ltd., Xi'an, China).

Genotyping of SNPs

Ten candidate SNPs (rs112276562, rs6790894, rs6551121, rs6551122, rs1907168, rs73150416, rs12635768, rs6551130, rs78205284 and rs6788033) in *LRRC3B* was selected according to the 1000 Genomes Project data, with the minor allele frequency (MAF) ≥ 0.05 in Chinese Han population and a pairwise $r^2 > 0.80$. *LRRC3B* SNPs were genotyped by MassARRAY platform (Agena Bioscience, San Diego, CA, USA) [16, 17]. The primers were shown in Supplementary Table 1. About 10% of the samples were randomly selected and genotyped in duplicate for quality control, and the concordance were 100%.

In silico analysis

Prediction of *LRRC3B* SNPs possible function was performed with HaploReg v4.1 online tool. With the purpose to assess *LRRC3B* mRNA expression in BC, GEPIA database was used. Kaplan–Meier plotter was used to evaluate the prognosis of *LRRC3B* expression in BC.

Data analysis

Student's t test was performed to evaluate the age distribution. Hardy-Weinberg equilibrium (HWE) was tested by a goodness-of-fit χ^2 test in controls. The genotype frequencies of *LRRC3B* SNPs between patients and controls were analyzed by χ^2 test. Odds ratios (OR) and 95% confidence interval (CI) adjusted age were calculated, and haplotype frequencies were estimated using PLINK software. Pairwise linkage disequilibrium (LD) analysis was analyzed using Haploview software (version 4.2). *p*-values < 0.05 were considered significant.

Results

The features of all subjects were summarized in Table 1. 563 BC patients (52.05 ± 9.81 years) and 552 healthy controls (51.88 ± 9.85 years) participated in the present study. No significant differences of age distribution between cases and controls ($p = 0.767$) was found. Most patients had tumor size > 2 cm (56.0%), stage I/II (64.8%), lymph node invasion (48.8%), positive hormone receptor status such as ER + (67.1%), PR + (60.6%), or HER2 + (48.5%) and positive Ki67 (64.8%).

Table 1
Characteristics of patients with breast cancer and controls

Characteristics		Cases (n = 563)	Controls (n = 552)	<i>p</i>
Age	Mean ± SD (years)	52.05 ± 9.81	51.88 ± 9.85	0.767
	> 51	297 (52.8%)	295 (53.4%)	
	≤ 51	266 (47.2%)	257 (46.6%)	
Tumor size (cm)	≤ 2 cm	107 (19.0%)		
	> 2 cm	315 (56.0%)		
	Missing	141 (25.0%)		
Stage	I-II	365 (64.8%)		
	III-IV	162 (28.8%)		
	Missing	36 (6.4%)		
Lymph node invasion	No	260 (46.2%)		
	Yes	275 (48.8%)		
	Missing	28 (5.0%)		
ER status	Negative	161 (28.6%)		
	Positive	378 (67.1%)		
	Missing	24 (4.3%)		
PR status	Negative	212 (37.7%)		
	Positive	341 (60.6%)		
	Missing	10 (1.8%)		
HER2 status	Negative	91 (16.2%)		
	Positive	273 (48.5%)		
	Missing	199 (35.3%)		
Ki67	Negative	132 (23.4%)		
	Positive	365 (64.8%)		
	Missing	66 (11.7%)		
ER, estrogen receptor; RP, progesterone receptor; HER2, human epidermal growth factor receptor 2.				

The MAF of the *LRRC3B* SNPs were more than 0.05 (Supplementary Table 2). No deviation from the HWE for the polymorphisms examined was observed in the genotype distributions of the controls ($p > 0.05$), suggesting these subjects could represent the general population. In addition, *LRRC3B* variants were related to enhancer histone, changed motifs and GRASP QTL hits *in silico* analysis.

We performed allele and genetic model analyses of ten *LRRC3B* SNPs in the BC cases and control subjects. The genotype frequencies distribution of SNPs was shown in Table 2 and Supplementary Table 3. The distributions of A and T alleles of *LRRC3B* rs1907168 polymorphism were 84.6% and 15.4% in controls and 87.7% and 12.3% in patients. An association between T allele and BC risk (OR = 0.77, 95% CI: 0.61–0.99, $p = 0.037$) was found. Similarly, rs1907168 had a decreased risk of BC (heterozygous: OR = 0.71, 95% CI: 0.54–0.94, $p = 0.017$; dominant: OR = 0.72, 95% CI: 0.55–0.95, $p = 0.019$; and log-additive: OR = 0.76, 95% CI: 0.59–0.97, $p = 0.030$). However, no significant association was found for other SNPs (Supplementary Table 3).

Table 2
Relationships between *LRRC3B* rs1907168 polymorphism and breast cancer risk

Model	Genotype	Case	Control	Adjusted by age and gender	
				OR (95%CI)	p
Allele	A	987	929	1.00	0.037
	T	139	169	0.77 (0.61–0.99)	
Genotype	AA	432	388	1.00	0.017
	AT	123	153	0.71 (0.54–0.94)	
	TT	8	8	0.90 (0.33–2.42)	
Dominant	AA	432	388	1.00	0.019
	AT-TT	131	161	0.72 (0.55–0.95)	
Recessive	AA-AT	555	541	1.00	0.963
	TT	8	8	0.98 (0.36–2.62)	
Log-additive	—	—	—	0.76 (0.59–0.97)	0.030
SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval.					
<i>p</i> values were calculated by logistic regression analysis with adjustments for age and gender.					
<i>p</i> < 0.05 means the data is statistically significant.					

We further conducted subgroup analyses by age to examine the effects of the *LRRC3B* SNPs on BC risk (Table 3). *LRRC3B* rs1907168 reduced the risk of BC among young participants (age < 51 years) under the allele (OR = 0.69, 95% CI: 0.48–0.99, $p = 0.043$), heterozygote (OR = 0.53, 95% CI: 0.35–0.80, $p = 0.002$), dominant (OR = 0.58, 95% CI: 0.39–0.86, $p = 0.008$) and log-additive (OR = 0.68, 95% CI: 0.47–0.98, $p = 0.038$) models. In addition, rs78205284 polymorphism (TT vs. GG, OR = 2.83, 95% CI: 1.08–7.41, $p = 0.034$; and TT vs. GG-GT, OR = 2.72, 95% CI: 1.05–7.07, $p = 0.040$) contributed to the increased BC risk at age ≤ 51 years.

Table 3

Relationships between *LRRC3B* polymorphisms and breast cancer risk according to the stratification by age

SNP ID	Model	Genotype	> 51				≤ 51			
			Case	Control	OR (95%CI)	<i>p</i>	Case	Control	OR (95%CI)	<i>p</i>
rs1907168	Allele	A	515	500	1.00	0.336	472	429	1.00	0.043
		T	70	90	0.85 (0.62–1.18)		60	79	0.69 (0.48–0.99)	
	Genotype	AA	220	211	1.00	0.609	212	177	1.00	0.002
		AT	75	78	0.91 (0.63–1.32)		48	75	0.53 (0.35–0.80)	
		TT	2	6	0.32 (0.06–1.59)		6	2	2.54 (0.51–12.77)	
	Dominant	AA	220	211	1.00	0.437	212	177	1.00	0.008
		AT-TT	77	84	0.87 (0.60–1.25)		54	77	0.58 (0.39–0.86)	
	Recessive	AA-AT	295	289	1.00	0.172	260	252	1.00	0.188
		TT	2	6	0.33 (0.07–1.63)		6	2	2.95 (0.59–14.77)	
	Log-additive	—	—	—	0.83 (0.59–1.17)	0.287	—	—	0.68 (0.47–0.98)	0.038
rs78205284	Allele	G	482	477	1.00	0.993	419	428	1.00	0.063
		T	110	109	1.00 (0.74–1.34)		113	86	1.34 (0.98–1.83)	
	Genotype	GG	201	197	1.00	0.790	169	177	1.00	0.505
		GT	80	83	0.95 (0.66–1.37)		81	74	1.14 (0.78–1.66)	
		TT	15	13	1.15 (0.53–2.48)		16	6	2.83 (1.08–7.41)	
	Dominant	GG	201	197	1.00	0.900	169	177	1.00	0.207
		GT-TT	95	96	0.98 (0.69–1.38)		97	80	1.26 (0.88–1.82)	

SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval.

p values were calculated by logistic regression analysis with adjustments for age.*p* < 0.05 indicates statistical significance.

Recessive	GG-GT	281	280	1.00	0.694	250	251	1.00	0.040
	TT	15	13	1.17 (0.54- 2.50)		16	6	2.72 (1.05- 7.07)	
Log-additive	—	—	—	1.01 (0.76- 1.33)	0.966			1.33 (0.98- 1.80)	0.071

SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval.

p values were calculated by logistic regression analysis with adjustments for age.

p < 0.05 indicates statistical significance.

We also investigated the potential associations of *LRRC3B* polymorphisms with clinicopathological characteristics of BC, including tumor size, lymph node metastasis, clinical stage, the statuses of ER, PR, Her-2, and Ki67 (Table 4). We found that rs6551122 (OR = 0.51, *p* = 0.028) and rs12635768 (homozygote, OR = 0.36, *p* = 0.023; recessive, OR = 0.39, *p* = 0.032; log-additive, OR = 0.69, *p* = 0.043) polymorphisms were related to a smaller BC tumor size (< 2 cm). In addition, rs112276562, rs6551122 and rs73150416 variants contributed to a lower incidence of PR-positive breast cancer (for rs112276562, heterozygote OR = 0.56, *p* = 0.002 and dominant OR = 0.63, *p* = 0.011; for rs6551122, heterozygote OR = 0.63, *p* = 0.016 and dominant OR = 0.64, *p* = 0.018; and for rs73150416, heterozygote OR = 0.57, *p* = 0.005, dominant OR = 0.61, *p* = 0.008, log-additive OR = 0.72, *p* = 0.035, and allele OR = 0.71, *p* = 0.029). Moreover, rs6788033 was associated with a lower the expression level of Ki-67in dominant (OR = 0.64, *p* = 0.030), log-additive (OR = 0.68, *p* = 0.024) and allele (OR = 0.69, *p* = 0.025) models. There was no significant association of *LRRC3B* polymorphisms with respect to the other variables like lymph node metastasis, clinical stage and ER/HER2 status.

Table 4
The association between *LRRC3B* polymorphisms and clinical features of breast cancer

SNPs ID	Variables		OR (95%), <i>p</i>					
			Homozygote	Heterozygote	Dominant	Recessive	Log-additive	Allele
rs112276562	Tumour size	< 2 cm/ ≥ 2 cm	0.90 (0.31–2.64), 0.847	1.39 (0.83–2.34), 0.212	1.31 (0.80–2.13), 0.281	0.82 (0.28–2.40), 0.721	1.17 (0.78–1.76), 0.441	1.16 (0.77–1.75), 0.486
	LNM	(-)/ (+)	0.61 (0.25–1.47), 0.272	0.81 (0.56–1.18), 0.271	0.78 (0.55–1.12), 0.183	0.65 (0.27–1.56), 0.337	0.80 (0.59–1.08), 0.145	0.80 (0.59–1.08), 0.145
	Stage	II-II/III-IV	0.75 (0.29–1.96), 0.560	0.81 (0.54–1.23), 0.326	0.80 (0.54–1.20), 0.282	0.80 (0.31–2.07), 0.648	0.83 (0.60–1.17), 0.289	0.84 (0.60–1.18), 0.310
	ER	(-)/ (+)	1.78 (0.59–5.40), 0.308	0.71 (0.48–1.06), 0.090	0.78 (0.53–1.14), 0.200	2.00 (0.66–6.00), 0.218	0.90 (0.65–1.24), 0.524	0.90 (0.65–1.24), 0.517
	PR	(-)/ (+)	1.54 (0.59–4.03), 0.375	0.56 (0.39–0.81), 0.002	0.63 (0.44–0.90), 0.011	1.87 (0.72–4.84), 0.196	0.77 (0.57–1.04), 0.093	0.77 (0.57–1.04), 0.085
	HER2	(-)/ (+)	0.88 (0.27–2.91), 0.835	1.30 (0.76–2.22), 0.339	1.24 (0.74–2.06), 0.410	0.81 (0.25–2.66), 0.732	1.14 (0.74–1.76), 0.561	1.11 (0.72–1.72), 0.626
	Ki-67	< 10%/ ≥ 10%	1.17 (0.41–3.34), 0.768	1.06 (0.69–1.64), 0.790	1.07 (0.70–1.63), 0.743	1.15 (0.41–3.24), 0.794	1.07 (0.75–1.53), 0.713	1.06 (0.74–1.51), 0.761
rs6551122	Tumour size	< 2 cm/ ≥ 2 cm	0.59 (0.30–1.13), 0.109	1.32 (0.81–2.14), 0.268	1.08 (0.69–1.69), 0.734	0.51 (0.28–0.93), 0.028	0.87 (0.63–1.20), 0.393	0.88 (0.64–1.21), 0.420
	LNM	(-)/ (+)	0.76 (0.44–1.30), 0.312	0.92 (0.64–1.33), 0.670	0.88 (0.62–1.26), 0.492	0.79 (0.48–1.31), 0.359	0.88 (0.69–1.14), 0.337	0.89 (0.70–1.14), 0.367
	Stage	II-II/III-IV	0.66 (0.36–1.23), 0.194	1.06 (0.71–1.57), 0.794	0.96 (0.65–1.40), 0.825	0.64 (0.36–1.15), 0.135	0.88 (0.67–1.16), 0.355	0.89 (0.68–1.16), 0.389

SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; LNM, lymph node metastasis.

p values were calculated by logistic regression analysis with adjustments for age.

p < 0.05 indicates statistical significance.

	ER	(-)/ (+)	1.05 (0.58– 1.92), 0.866	0.84 (0.56– 1.25), 0.380	0.88 (0.60– 1.29), 0.504	1.17 (0.67– 2.03), 0.582	0.97 (0.74– 1.28), 0.842	0.98 (0.75– 1.28), 0.866
	PR	(-)/ (+)	0.71 (0.41– 1.23), 0.218	0.63 (0.43– 0.92), 0.016	0.64 (0.45– 0.93), 0.018	0.93 (0.57– 1.53), 0.776	0.79 (0.61– 1.02), 0.064	0.80 (0.62– 1.03), 0.078
	HER2	(-)/ (+)	0.96 (0.42– 2.17), 0.918	0.83 (0.49– 1.39), 0.475	0.85 (0.52– 1.40), 0.524	1.07 (0.50– 2.28), 0.860	0.93 (0.65– 1.34), 0.699	0.93 (0.66– 1.32), 0.690
	Ki-67	< 10%/ ≥ 10%	1.32 (0.69– 2.52), 0.408	1.38 (0.90– 2.13), 0.140	1.37 (0.91– 2.06), 0.133	1.10 (0.60– 2.02), 0.757	1.21 (0.90– 1.65), 0.212	1.21 (0.90– 1.63), 0.203
rs73150416	Tumour size	< 2 cm/ ≥ 2 cm	0.71 (0.26– 1.95), 0.505	1.22 (0.71– 2.07), 0.473	1.11 (0.68– 1.82), 0.676	0.67 (0.25– 1.85), 0.444	1.01 (0.68– 1.51), 0.953	0.99 (0.66– 1.50), 0.972
	LNM	(-)/ (+)	0.54 (0.22– 1.34), 0.183	0.79 (0.54– 1.16), 0.226	0.75 (0.52– 1.09), 0.130	0.58 (0.24– 1.42), 0.232	0.77 (0.56– 1.04), 0.091	0.76 (0.55– 1.04), 0.087
	Stage	II–III/III–IV	0.47 (0.16– 1.43), 0.183	0.84 (0.55– 1.28), 0.405	0.78 (0.52– 1.18), 0.236	0.50 (0.16– 1.49), 0.211	0.78 (0.55– 1.10), 0.151	0.78 (0.54– 1.11), 0.159
	ER	(-)/ (+)	1.29 (0.46– 3.60), 0.634	0.75 (0.50– 1.13), 0.169	0.80 (0.54– 1.18), 0.261	1.40 (0.50– 3.89), 0.519	0.89 (0.64– 1.23), 0.474	0.88 (0.63– 1.24), 0.464
	PR	(-)/ (+)	0.95 (0.39– 2.34), 0.916	0.57 (0.39– 0.84), 0.005	0.61 (0.42– 0.88), 0.008	1.13 (0.46– 2.74), 0.792	0.72 (0.53– 0.98), 0.035	0.71 (0.52– 0.97), 0.029
	HER2	(-)/ (+)	1.26 (0.34– 4.66), 0.730	1.11 (0.65– 1.89), 0.704	1.13 (0.67– 1.88), 0.652	1.22 (0.33– 4.48), 0.764	1.11 (0.72– 1.72), 0.628	1.09 (0.70– 1.69), 0.700
	Ki-67	< 10%/ ≥ 10%	1.01 (0.38– 2.67), 0.987	1.03 (0.66– 1.62), 0.895	1.03 (0.67– 1.58), 0.900	1.00 (0.38– 2.62), 0.999	1.02 (0.72– 1.45), 0.918	1.01 (0.70– 1.45), 0.966
rs12635768	Tumour size	< 2 cm/ ≥ 2 cm	0.36 (0.15– 0.87), 0.023	0.81 (0.50– 1.30), 0.378	0.72 (0.46– 1.12), 0.144	0.39 (0.16– 0.92), 0.032	0.69 (0.48– 0.99), 0.043	0.71 (0.50– 1.01), 0.058

SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval; ER, estrogen receptor; RP, progesterone receptor; HER2, human epidermal growth factor receptor 2; LNM, lymph node metastasis.

p values were calculated by logistic regression analysis with adjustments for age.

p < 0.05 indicates statistical significance.

	LNM	(-)/ (+)	1.27 (0.59– 2.71), 0.541	1.11 (0.77– 1.59), 0.577	1.13 (0.8– 1.59), 0.495	1.22 (0.58– 2.57), 0.605	1.12 (0.84– 1.48), 0.448	1.12 (0.84– 1.48), 0.434
	Stage	II-III/III-IV	1.32 (0.58– 2.98), 0.506	1.16 (0.79– 1.72), 0.454	1.18 (0.81– 1.72), 0.386	1.24 (0.56– 2.76), 0.594	1.16 (0.85– 1.57), 0.359	1.15 (0.85– 1.56), 0.364
	ER	(-)/ (+)	0.88 (0.38– 2.01), 0.759	0.92 (0.62– 1.35), 0.660	0.91 (0.63– 1.33), 0.627	0.91 (0.40– 2.05), 0.818	0.93 (0.68– 1.26), 0.625	0.93 (0.69– 1.27), 0.658
	PR	(-)/ (+)	0.96 (0.44– 2.09), 0.910	0.87 (0.60– 1.25), 0.448	0.88 (0.62– 1.25), 0.470	1.01 (0.47– 2.18), 0.981	0.92 (0.69– 1.22), 0.557	0.93 (0.70– 1.24), 0.628
	HER2	(-)/ (+)	1.11 (0.35– 3.51), 0.857	0.71 (0.43– 1.16), 0.173	0.75 (0.46– 1.21), 0.233	1.27 (0.41– 3.95), 0.675	0.85 (0.57– 1.26), 0.407	0.86 (0.58– 1.27), 0.447
	Ki-67	< 10%/ ≥ 10%	1.80 (0.59– 5.44), 0.300	1.06 (0.69– 1.62), 0.786	1.12 (0.74– 1.69), 0.589	1.76 (0.59– 5.26), 0.314	1.16 (0.82– 1.64), 0.411	1.18 (0.84– 1.67), 0.345
rs6788033	Tumour size	< 2 cm/ ≥ 2 cm	1.12 (0.43– 2.96), 0.813	1.29 (0.81– 2.07), 0.282	1.27 (0.81– 1.99), 0.297	1.02 (0.39– 2.64), 0.973	1.18 (0.81– 1.72), 0.384	1.19 (0.82– 1.72), 0.360
	LNM	(-)/ (+)	0.56 (0.24– 1.32), 0.183	1.02 (0.71– 1.45), 0.924	0.95 (0.68– 1.35), 0.791	0.56 (0.24– 1.29), 0.172	0.90 (0.67– 1.20), 0.474	0.90 (0.68– 1.20), 0.473
	Stage	II-III/III-IV	0.33 (0.10– 1.14), 0.079	1.02 (0.69– 1.50), 0.941	0.92 (0.63– 1.35), 0.685	0.33 (0.10– 1.12), 0.076	0.85 (0.61– 1.17), 0.317	0.85 (0.62– 1.16), 0.302
	ER	(-)/ (+)	1.39 (0.54– 3.58), 0.500	1.02 (0.69– 1.50), 0.933	1.05 (0.72– 1.53), 0.795	1.38 (0.54– 3.52), 0.503	1.08 (0.78– 1.48), 0.645	1.07 (0.78– 1.47), 0.660
	PR	(-)/ (+)	1.72 (0.70– 4.26), 0.239	1.11 (0.77– 1.60), 0.572	1.16 (0.82– 1.65), 0.401	1.66 (0.68– 4.05), 0.269	1.19 (0.88– 1.60), 0.268	1.17 (0.88– 1.57), 0.286
	HER2	(-)/ (+)	0.52 (0.17– 1.62), 0.259	0.78 (0.47– 1.28), 0.325	0.75 (0.46– 1.21), 0.234	0.57 (0.19– 1.76), 0.330	0.75 (0.50– 1.13), 0.175	0.78 (0.53– 1.16), 0.215

SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; LNM, lymph node metastasis.

p values were calculated by logistic regression analysis with adjustments for age.

p < 0.05 indicates statistical significance.

Ki-67	< 10%/ ≥ 10%	0.50 (0.21–1.20), 0.119	0.66 (0.44–1.01), 0.054	0.64 (0.43–0.96), 0.030	0.59 (0.25–1.39), 0.225	0.68 (0.49–0.95), 0.024	0.69 (0.50–0.95), 0.025
SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval; ER, estrogen receptor; RP, progesterone receptor; HER2, human epidermal growth factor receptor 2; LNM, lymph node metastasis.							
<i>p</i> values were calculated by logistic regression analysis with adjustments for age.							
<i>p</i> < 0.05 indicates statistical significance.							

Figure 1 represented the reconstructed LD plot, and had three blocks in *LRRC3B* SNPs (Block 1, rs112276562 and rs6790894; Block 2, rs6551121, rs6551122, rs1907168 and rs73150416; and Block 3, rs12635768, rs6551130, rs78205284 and rs6788033). Moreover, ‘GATT’ haplotype in the Block 2 had a higher risk for BC (OR = 1.29, 95% CI: 1.00–1.65, *p* = 0.048, Supplementary Table 4).

According to GEPIA database, *LRRC3B* gene was down-regulated in BC (*p* < 0.01, Supplementary Fig. 1). Kaplan–Meier plotter showed high expression of *LRRC3B* was related to better BC overall survival (HR = 0.83, 95% CI: 0.71–0.97, *p* = 0.017, Supplementary Fig. 2). We speculated that *LRRC3B* is a tumor suppressor gene, but more convincing studies are needed to validate the conclusions because these clinical data are from the database.

Discussion

This study investigated the effect of *LRRC3B* genetic variation on BC risk in a Chinese Han population. The result revealed that rs1907168 conferred to reduced BC risk overall. In additional, rs1907168 decreased the risk of BC while rs78205284 increased BC susceptibility among the population at age ≤ 51 years. Noticeably, clinical parameters such as the status of PR and Ki67 and tumor size were significantly associated with *LRRC3B* polymorphism. Furthermore, we found that the existence of three blocks in *LRRC3B* SNPs and the association of ‘GATT’ haplotype with an increased BC risk. Our results suggested *LRRC3B* variants might be susceptibility markers for BC. Specially, this is firstly demonstrated the effects of *LRRC3B* SNPs on BC susceptibility.

LRRC3B, located at 3p24, contained a leucine-rich repeat N-terminal domain (Leu, >13%). The protein-conserved region of *LRRC3B* exists cAMP- and cGMP-dependent protein kinase phosphorylation sites; thus, related to cell differentiation, cycle regulation, proliferation and invasion [18, 19]. Several studies have revealed *LRRC3B* could be as a tumor suppressor in carcinogenesis [20, 21]. Formerly, the expression of *LRRC3B* was reduced in BC tissues and associated with tumor grade of BC [13]. Consistently, we also found that *LRRC3B* was down-regulated in BC and low expression of *LRRC3B* was related to a poor overall survival for BC based on bioinformatics analysis. Moreover, *LRRC3B* might serve a protective role in preventing bupivacaine-induced BC recurrence and metastasis[15]. These results hint us that *LRRC3B* has an important role in BC occurrence and progression.

Genetic variations in *LRRC3B* might be associate with the development and progress of disease. However, there is no finding of the related research about *LRRC3B* polymorphisms. In this study, *LRRC3B* rs1907168 (A/T) variant have a protective effect in BC risk. Further, stratified analysis showed that rs1907168 decreased while rs78205284 increased BC susceptibility among the population at age ≤ 51 years. Notably, the peak age for BC is 40–50 years in the Asian countries, whereas 60–70 years in the Western countries [22]. This finding suggested that age might affect the association of genetic variations and BCC, and that rs78205284 was a risk factor to BC among younger women in Chinese Han population.

As BC is a complex disease, the same variant might show a different response for BC risk across different tumor size, clinical stage and clinical features [23, 24]. We further evaluated association of *LRRC3B* polymorphisms and clinical features of BC, including tumor size, lymph node metastasis, clinical stage, the statuses of ER, PR, Her-2, and Ki67. We found that rs6551122 and rs12635768 polymorphisms were associated with a smaller BC tumor size (< 2 cm). In addition, rs112276562, rs6551122 and rs73150416 variants contributed to a lower incidence of PR-positive BC, suggesting that *LRRC3B* polymorphisms might involve in the pathogenesis of PR-positive BC. Moreover, rs6788033 was associated with a lower level of Ki-67. Our findings showed *LRRC3B* polymorphisms were associated with tumors size and PR / Ki-67 status, which might act as predictors for BC occurrence and development.

Our study provided the evidence that *LRRC3B* variants were associated with BC susceptibility and related to clinical characteristic of BC patients. Inevitably, there were several intrinsic limitations in this study. First, data on *LRRC3B* SNPs potential function, and *LRRC3B* expression/survival in BC were predicted *in silico* analysis only; thus, further functional assay is necessary to explore the functions and underlying mechanisms of these polymorphisms. Second, gene-to-environment interactions could not be further analyzed due to the unavailability of relevant information; therefore, additional studies will be required.

Conclusion

In conclusion, we firstly reported that *LRRC3B* polymorphisms might contribute to individual susceptibility and progression to BC among Chinese Han females. The study might help to understand the possible effect of *LRRC3B* variants in the development of BC.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Xizang Minzu University and in accordance with the 1964 Helsinki Declaration. Written informed consent was obtained.

Consent for publication

Not applicable.

Availability of data and material

All the data regarding the findings are available within the manuscript.

Competing interests

The authors declare that they have no conflict of interest.

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No.

Authors' contributions

Tianbo Jin: Writing - original draft, Conceptualization. Linna Peng, Shishi Xing, and Dandan Li: Methodology. Chunjuan He and Xue He: Data curation. Li Wang: Conceptualization, Writing - review & editing.

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Figures

Figure 1

Haplotype block map for ten SNPs in the LRRC3B gene. The numbers inside the diamonds indicate the D' for pairwise analyses.

Supplementary Files

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