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Research Paper

HMGB1 genetic polymorphisms are biomarkers for the development and progression of breast cancer

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Abstract

Breast cancer is a major cause of cancer mortality worldwide. High-mobility group box protein 1 (HMGB1) is a ubiquitous nuclear protein found in all mammal eukaryotic cells that participates in tumor progression, migration and metastasis. HMGB1 overexpression has been indicated in breast cancer patients. However, scant information is available regarding the association between *HMGB1* single nucleotide polymorphisms (SNPs) and the risk or prognosis of breast cancer. We report on the association between 4 SNPs of the *HMGB1* gene (rs1360485, rs1045411, rs2249825 and rs1412125) and breast cancer susceptibility as well as clinical outcomes in 313 patients with breast cancer and in 217 healthy controls. Patients with one G allele in the rs1360485 or rs2249825 domains are likely to progress to T2 tumor and lymph node metastasis. In addition, the presence of one G allele in SNPs rs1360485 or rs2249825 was associated with a higher risk of progressing to T2 tumor and distant metastasis amongst HER2-enriched and triple-negative breast cancer (TNBC) tumors compared with luminal A and luminal B tumors. Furthermore, having one C allele in the rs1412125 domain increased the risk of pathologic grade 3 disease in HER2-enriched and TNBC tumors. Our results indicate that genetic variations in the *HMGB1* gene may serve as an important predictor of breast cancer progression and metastasis.

Key words: *HMGB1* polymorphisms; Breast cancer; Single nucleotide polymorphism; Susceptibility

Introduction

Breast cancer is associated with high mortality. Over a million women worldwide are diagnosed with breast cancer every year and over 500,000 succumb to the disease [1]. Risk factors associated with breast cancer in women include age, family history, reproductive and gynecologic factors, as well as lifestyle factors such as alcohol consumption and lack of physical activity, amongst others [2]. Women who are at high risk of breast cancer may be advised to maintain their mammography screening schedule, undergo genetic testing, or commence chemo-prevention.

Current statistical models for estimating breast cancer risk have limited sensitivity and specificity [2]. Researchers have therefore explored genetic variation associated with breast cancer risk, in order to

determine whether single nucleotide polymorphism (SNP) genotyping will more accurately stratify breast cancer risk and guide disease management. Emerging reports indicate an association between SNPs in certain genes and susceptibility to breast cancer, as well as clinicopathologic status. Besides the recognized *BRCA1* and *BRCA2* mutations that markedly increase the risk of developing breast cancer [3, 4], a number of additional low- and moderate-risk susceptibility variants have been identified, including caspase-8 (CASP8), an enzyme involved in apoptosis [5].

High-mobility group box protein 1 (HMGB1) is a ubiquitous nuclear protein that has been discovered in mammals [6, 7]. HMGB1 contains DNA binding domains and contributes to DNA repair and the stabilization of nuclear homeostasis [8]. HMGB1 is usually localized in the cell nucleus and is secreted into the extracellular environment in response to different stimuli; either passively during cellular apoptosis or necrosis, or actively following inflammatory signals from activated immune cells or neuronal cells [9]. It has been reported HMGB1 SNPs controls with rheumatoid arthritis disease outcome [10]. Previous research has confirmed the association of *HMGB1* SNPs with the susceptibility and progression of disease, such as hepatocellular carcinoma [11], lung cancer [12] and uterine cervical neoplasia [13]. An increase in HMGB1 levels in response to neoadjuvant chemotherapy has been found to be a prognostic marker of survival in early breast cancer patients [14] and recent research has demonstrated a cumulative impact of multiple risk-associated polymorphisms in the HMGB1/receptor for advanced glycation end products (HMGB1/RAGE) pathway upon breast cancer progression [15]. However, the association between *HMGB1* SNPs and breast cancer risk, prognosis, metastasis and clinical aspects is unclear. We therefore conducted a case-control study to evaluate the role of *HMGB1* SNPs in breast cancer susceptibility and clinicopathologic features in a cohort of Chinese Han individuals.

Materials and Methods

Participants

Between 2014 and 2016, we collected 313 blood specimens from patients (cases) who had been diagnosed with breast cancer at Dongyang People's Hospital. The control group consisted of 217 healthy participants without a history of cancer. All participants provided written informed consent, and the study was approved by the Ethics Committee of Dongyang People's Hospital. Pathohistologic diagnosis followed the World Health Organization classification of breast tumors and tumors were graded using the Scarff-Bloom-Richardson method [16]. Breast cancer cases were categorized by estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and Ki-67 status into 4 subtypes : Luminal A (ER^+ and/or PR^+, $HER2^-$, Ki-67 <14%); Luminal B (ER^+ and/or PR^+, $HER2^-$, Ki-67 ≥14%; or ER^+ and/or PR^+, $HER2^+$); HER2-enriched (ER^-, PR^-, $HER2^+$); or TNBC (ER^-, PR^-, $HER2^-$) [17-19]. Demographic data on age, sex, smoking history and alcohol consumption were obtained from a standardized questionnaire and electronic medical records.

SNP selection

SNP rs2249825 (3814C/G; genomic number 31,037,903) near the exon, rs1360485 (3′UTR, T/C; genomic number 31,031,884) in the 3′ untranslated region, SNP rs1412125 (-1615T/C; genomic number 31,041,595) in the promoter region and rs1045411 (2262C/T; genomic number 31,033,232) in the exon were selected according to Chinese HapMap data and previous studies [13, 20]. The minor allele frequencies of these SNPs were all ≥5 %.

Determination of genotypes

Total genomic DNA was isolated from whole blood specimens using QIAamp DNA blood mini kits (Qiagen, Valencia, CA), as per the manufacturer's instructions. DNA was dissolved in a Tris-EDTA (TE) buffer containing 10 mM Tris-HCl, 1 mM EDTA Na_2 (pH 7.8) and stored at $^-$20°C until it was subjected to quantitative polymerase chain reaction (PCR) analysis. Four *HMGB1* SNPs (rs1360485, rs1045411, rs2249825 and rs1412125) were examined with the use of a commercially available TaqMan SNP genotyping assay (Applied Biosystems, Warrington, UK), according to the manufacturer's protocols [21, 22].

Statistical analysis

The genotype distribution of each SNP was analyzed for Hardy–Weinberg equilibrium and confirmed by Chi-square analysis. Demographic characteristics were compared between patients and controls using the Mann–Whitney U-test and Fisher's exact test. Associations between genotypes, breast cancer risk and clinicopathologic characteristics were estimated using adjusted odds ratios (AORs) and 95% confidence intervals (CIs), after controlling for other covariates. Significant differences in haplotype frequencies between cases and controls were analyzed using Haploview, according to the software package [23]. A p value of < 0.05 was considered statistically significant. Data were analyzed using SAS statistical software (Version 9.1, 2005; SAS Institute Inc., Cary, NC).

Results

Sociodemographic characteristics and clinical parameters for all study participants are shown in Table 1. Significant between-group differences were observed for age, tobacco use and alcohol consumption ($p < 0.05$). Most patients (76.7%) had stage I/II breast cancer; 23.3% had stage III/IV disease (Table 1). In addition, the majority of patients were ER-negative (69.6%) or HER2-positive (63.6%) (Table 1).

Table 1. Baseline demographic and clinical characteristics of the study population.

Variable	Controls (n=217) N (%)	Patients (n=313) N (%)	*p* value
Age (years)	**Mean ± S.D.**	**Mean ± S.D.**	
	43.4±17.1	53.2±11.4	< 0.001*
Alcohol consumption			
No	176 (81.1)	294 (93.9)	< 0.05
Yes	41 (18.9)	19 (6.1)	
Tobacco consumption			
No	187 (86.2)	311 (99.4)	< 0.05
Yes	30 (13.8)	2 (0.6)	
Clinical stage			
I-II		240 (76.7)	
III-IV		73 (23.3)	
Tumor T status			
≤T2		297 (94.9)	
>T2		16 (5.1)	
Lymph node status			
N0		160 (51.1)	
>N0		153 (48.9)	
Distant metastasis			
M0		303 (96.8)	
M1		10 (3.2)	
Histologic grade			
G1+G2		187 (59.7)	
G3		125 (39.9)	
ER status			
Positive		95 (30.4)	
Negative		218 (69.6)	
PR status			
Positive		144 (46)	
Negative		169 (54)	
HER2			
Positive		199 (63.6)	
Negative		114 (36.4)	

S.D. = standard deviation; T = primary tumor; T1 = tumor ≤5 cm; T2 = tumor >5 cm; N0 = no regional lymph node metastasis; M0 = no clinical or radiographic evidence of distant metastasis; M1 = distant detectable metastases as determined by classic clinical and radiographic means and/or histologically proven >0.2 mm; G1 = well differentiated; G2 = moderately differentiate; G3 = poorly differentiated; ER = estrogen receptor; PR = progesterone receptor; HER2 = human epidermal growth factor receptor 2.
The Mann-Whitney U test or Fisher's exact test was used to compare values between controls and patients with breast cancer. * p value < 0.05 was considered statistically significant.

HMGB1 genotype distribution patterns for all participants are shown in Table 2. In the healthy controls, all genotypic frequencies were in Hardy–Weinberg equilibrium ($p > 0.05$). In both patients and controls, most of those with the rs1360485, rs1045411, rs2249825 and rs1412125 SNPs were, respectively, homozygous for A/A, homozygous for G/G, homozygous for C/C, and homozygous for T/T alleles (Table 2). In analyses adjusted for potential confounders, there were no significant differences between cases and controls in regard to the frequency of each of the 4 studied polymorphisms (Table 2).

Next, we compared the distributions of clinical aspects and *HMGB1* genotypes amongst cases. We found that patients with one G allele in the rs1360485 SNP (AOR 2.466; 95% CI: 1.068-5.694), one G allele in the rs2249825 SNP (AOR 3.264; 95% CI: 1.330-8.011), or one C allele in the rs1412125 SNP (AOR 2.702; 95% CI: 1.181-6.182) were more likely to progress to T2 breast cancer (Table 3). Patients with one G allele in the rs1360485 SNP (AOR 1.444; 95% CI: 0.944-2.207), one A allele in the rs1045411 (AOR 1.443; 95% CI: 0.935-2.228, or one G allele in the rs2249825 (AOR 1.515; 95% CI: 0.937-2.448) were at increased risk of developing lymph node metastasis disease (N2+N3) (Table 3).

In an analysis of clinical aspects and *HMGB1* genotypic frequencies in different breast cancer subtypes, we found no significant differences between cases and controls in regard to the frequency of *HMGB1* polymorphisms (Table 4).

In HER2 and TNBC subtypes, patients with one G allele in the rs1360485 SNP (AOR 6.061; 95% CI: 2.190-16.774), one A allele in the rs1045411 SNP (AOR 3.321; 95% CI: 1.216-9.068), one G allele in the rs2249825 SNP (AOR 5.800; 95% CI: 2.098-16.033), or one C allele in the rs1412125 SNP (AOR 5.849; 95% CI: 2.116-16.165) were likely to progress to T2 breast cancer (Table 5). Individuals with one G allele in the rs1360485 SNP (AOR 4.918; 95% CI: 1.479-16.353), or one A allele in the rs1045411 SNP (OR 5.847; 95% CI: 1.749-19.551) were likely to progress to distant metastatic disease (Table 5). Furthermore, the presence of one C allele in the rs1412125 SNP (AOR 2.112; 95% CI: 1.028-4.341) increased the likelihood of developing pathologic grade (G3) disease (Table 5).

Discussion

HMGB1 plays multiple roles inside and outside cells, such as chromatin stabilization, DNA repair, gene transcription, program cell death regulation, and immune response. The *HMGB1* gene has been implicated in tumor progression in various types of cancer such as colon, liver, breast, oral, and lung cancer [11, 24-26]. Previous research has indicated that *HMGB1* plays a role in breast cancer progression and metastasis [14, 27] and that inhibiting HMGB1 expression with quercetin promotes apoptosis in human breast adenocarcinoma cells [28]. These results suggest that HMGB1 knockdown might be a valuable therapeutic strategy for breast cancer.

Table 2. Distribution frequencies of *HMGB1* genotypes and 4 SNP alleles in controls and patients with breast cancer.

Variable	Controls (n=217) N (%)	Patients (n=313) N (%)	OR (95% CI)	*p* value	AOR[a] (95% CI)	*p* value
rs1360485						
AA	131 (60.4)	191 (61.0)	1.00 (reference)		1.00 (reference)	
AG	71 (32.7)	99 (31.6)	0.956 (0.656-1.395)	0.82	0.947 (0.636-1.412)	0.79
GG	15 (6.9)	23 (7.3)	1.052 (0.529-2.091)	0.89	1.020 (0.496-2.098)	0.96
AA	131 (60.4)	191 (61.0)	1.00 (reference)		1.00 (reference)	
AG+GG	86 (39.6)	122 (39.0)	0.973 (0.683-1.387)	0.88	0.949 (0.654-1.378)	0.78
A	333 (76.7)	481 (76.8)	1.00 (reference)		1.00 (reference)	
G	101 (23.3)	145 (23.2)	0.994 (0.744-1.328)	0.97	0.995 (0.664-1.491)	0.98
rs1045411						
GG	132 (60.8)	200 (63.9)	1.00 (reference)		1.00 (reference)	
GA	75 (34.6)	90 (28.8)	0.792 (0.543-1.155)	0.23	0.763 (0.513-1.135)	0.18
AA	10 (4.6)	23 (7.3)	1.518 (0.700-3.293)	0.29	1.551 (0.677-3.558)	0.3
GG	132 (60.8)	200 (63.9)	1.00 (reference)		1.00 (reference)	
GA+AA	85 (39.2)	113 (36.1)	0.877 (0.614-1.254)	0.47	0.845 (0.581-1.230)	0.38
G	339 (78.1)	490 (78.3)	1.00 (reference)		1.00 (reference)	
A	95 (21.9)	136 (21.7)	0.990 (0.736-1.332)	0.95	1.082 (0.708-1.653)	0.72
rs2249825						
CC	163 (75.1)	214 (68.4)	1.00 (reference)		1.00 (reference)	
CG	48 (22.1)	91 (29.1)	1.444 (0.963-2.164)	0.07	1.354 (0.885-2.070)	0.16
GG	6 (2.8)	8 (2.6)	1.016 (0.346-2.984)	0.98	1.015 (0.323-3.189)	0.98
CC	163 (75.1)	214 (68.4)	1.00 (reference)		1.00 (reference)	
CG+GG	54 (24.9)	99 (31.6)	1.396 (0.946-2.061)	0.09	1.313 (0.873-1.977)	0.19
C	374 (86.2)	519 (82.9)	1.00 (reference)		1.00 (reference)	
G	60 (13.8)	107 (17.1)	1.285 (0.912-1.811)	0.15	1.177 (0.737-1.879)	0.5
rs1412125						
TT	132 (60.8)	170 (54.3)	1.00 (reference)		1.00 (reference)	
TC	70 (32.3)	122 (39.0)	1.353 (0.933-1.962)	0.11	1.306 (0.884-1.931)	0.18
CC	15 (6.9)	21 (6.7)	1.087 (0.540-2.190)	0.82	1.131 (0.533-2.398)	0.75
TT	132 (60.8)	170 (54.3)	1.00 (reference)		1.00 (reference)	
TC+CC	85 (39.2)	143 (45.7)	1.306 (0.919-1.857)	0.14	1.266 (0.873-1.835)	0.21
T	334 (77)	462 (73.8)	1.00 (reference)		1.00 (reference)	
C	100 (23)	164 (26.2)	1.186 (0.891-1.578)	0.24	1.267 (0.851-1.885)	0.24

OR = odds ratio; AOR = adjusted odds ratio; CI = confidence interval.

[a] Logistic regression analysis adjusted for age, tobacco and alcohol consumption.

Table 3. Association of *HMGB1* alleles and 4 SNPs with the development and progression of breast cancer.

Allele	Patients (n=626) N (%)									
	Clinical stage		Tumor size		Lymph node metastasis		Distant metastasis		Pathologic grade	
	Stage I/II	Stage III/IV	≦T2	>T2	N0+N1	N2+N3	M0	M1	G1+G2	G3
rs1360485										
A	366 (76.1)	114 (78.6)	462 (96.0)	132 (91.0)	250 (52.0)	70 (48.3)	469 (97.5)	137 (94.5)	333 (69.5)	99 (68.3)
G	115 (23.9)	31 (21.4)	19 (4.0)	13 (9.0)	231 (48.0)	75 (51.7)	12 (2.5)	8 (5.5)	146 (30.5)	46 (31.7)
OR (95% CI)	1	0.865 (0.552-1.356)	1.00	**2.395 (1.152-4.977)***	1.00	1.160 (0.800-1.681)	1.00	2.282 (0.914-5.696)	1.00	1.060 (0.710-1.581)
AOR (95% CI)[a]	1	0.861 (0.513-1.446)	1.00	**2.466 (1.068-5.694)***	1.00	**1.444 (0.944-2.207)***	1.00	2.480 (0.824-7.458)	1.00	0.746 (0.464-1.199)
rs1045411										
G	369 (75.3)	111 (81.6)	467 (95.3)	127 (93.4)	254 (51.8)	66 (48;5)	477 (97.3)	129 (94.9)	337 (69.1)	95 (69.9)
A	121 (24.7)	25 (18.4)	23 (4.7)	9 (6.6)	236 (48.2)	70 (51.5)	13 (2.7)	7 (5.1)	151 (30.9)	41 (30.1)
OR (95% CI)	1	0.687 (0.425-1.110)	1.00	1.439 (0.650-3.187)	1.00	1.141 (0.781-1.669)	1.00	1.991 (0.778-5.093)	1.00	0.963 (0.637-1.456)
AOR (95% CI)	1	0.704 (0.406-1.221)	1.00	1.521 (0.625-3.700)	1.00	**1.443 (0.935-2.228)***	1.00	2.245 (0.741-6.804)	1.00	0.673 (0.412-1.098)
rs2249825										
C	395 (76.1)	85 (79.4)	498 (96.0)	96 (89.7)	271 (52.2)	49 (45.8)	504 (97.1)	102 (95.3)	359 (69.4)	73 (68.2)
G	124 (23.9)	22 (20.6)	21 (4.0)	11 (10.3)	248 (47.8)	58 (54.2)	15 (2.9)	5 (4.7)	158 (30.6)	34 (31.8)
OR (95% CI)	1	0.824 (0.495-1.374)	1.00	**2.717 (1.269-5.819)***	1.00	1.293 (0.8522-1.964)	1.00	1.647 (0.586-4.633)	1.00	1.058 (0..676-1.656)
AOR (95% CI)	1	0.860 (0.472-1.570)	1.00	**3.264 (1.330-8.011)***	1.00	**1.515 (0.937-2.448)***	1.00	2.159 (0.637-7.324)	1.00	0.827 (0.484-1.414)
rs1412125										
T	358 (77.5)	122 (74.4)	444 (96.1)	150 (91.5)	236 (51.1)	84 (51.2)	447 (96.8)	159 (97)	324 (70.3)	108 (66.3)
C	104 (22.5)	42 (25.6)	18 (3.9)	14 (8.5)	226 (48.9)	80 (48.8)	15 (3.2)	5 (3.0)	137 (297)	55 (33.7)
OR (95% CI)	1	1.185 (0.784-1.791)	1.00	**2.302 (1.118-4.742)***	1.00	0.995 (0.696-1.420)	1.00	0.937 (0.335-2.620)	1.00	1.204 (0.823-1.763)
AOR (95% CI)	1	1.370 (0.841-2.231)	1.00	**2.702 (1.181-6.182)***	1.00	1.086 (0.721-1.636)	1.00	1.145 (0.365-3.592)	1.00	1.170 (0.741-1.847)

HMGB1 = high-mobility group box protein 1; SNPs = single nucleotide polymorphisms; T2 = tumor >20 mm but ≤50 mm in greatest dimension; N0 = no regional lymph node metastasis; N1 = metastasis to movable ipsilateral level I, II axillary lymph node(s); N2 = metastases in ipsilateral level I, II axillary lymph nodes that are clinically fixed or matted or in clinically detected ipsilateral internal mammary nodes in the *absence* of clinically evident axillary lymph node metastasis; N3 = Metastases in ipsilateral infraclavicular (level III axillary) lymph node(s), with or without level I, II axillary node involvement, or in clinically detected ipsilateral internal mammary lymph node(s) and in the *presence* of clinically evident level I, II axillary lymph node metastasis; or metastasis in ipsilateral supraclavicular lymph node(s), with or without axillary or internal mammary lymph node involvement; M0 = no clinical or radiographic evidence of distant metastasis; M1 = distant detectable metastases as determined by classic clinical and radiographic means and/or histologically proven >0.2 mm; G1 = well differentiated; G2 = moderately differentiated; G3 = poorly differentiated.

[a] The odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were estimated using logistic regression adjusted for age, tobacco and alcohol consumption.

* p value < 0.05 was considered statistically significant.

Table 4. Allele frequencies of 4 *HMGB1* SNPs in controls and patients with breast cancer.

Allele	Luminal A + Luminal B				HER2 + TNBC		
Variable	Controls (n=434) N (%)	Patients (n=438) N (%)	OR (95% CI)	AOR (95% CI)	Patients (n=188) N (%)	OR (95% CI)	AOR (95% CI)
rs1360485							
A	333 (76.7)	339 (77.4)	1.00	0.963 (0.702-1.320)	142 (75.5)	1.00	1.068 (0.716-1.594)
G	101 (23.3)	99 (22.6)	1.00	0.948 (0.683-1.318)	46 (24.5)	1.00	1.022 (0.677-1.542)
rs1045411							
G	339 (78.1)	343 (78.3)	1.00	0.988 (0.717-1.363)	147 (78.2)	1.00	0.995 (0.658-1.506)
A	95 (21.9)	95 (21.7)	1.00	0.971 (0.695-1.358)	41 (21.8)	1.00	0.953 (0.623-1.459)
rs2249825							
C	374 (86.2)	365 (83.3)	1.00	1.247 (0.860-1.806)	154 (81.9)	1.00	1.376 (0.868-2.181)
G	60 (13.8)	73 (16.7)	1.00	1.188 (0.808-1.747)	34 (18.1)	1.00	1.284 (0.799-2.062)
rs1412125							
T	334 (77.0)	321 (73.3)	1.00	1.217 (0.895-1.656)	141 (75)	1.00	1.113 (0.747-1.659)
C	100 (23.0)	117 (26.7)	1.00	1.208 (0.876-1.667)	47 (25)	1.00	1.095 (0.726-1.652)

The odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were estimated using logistic regression models. AOR = adjusted odds ratio.

* p value < 0.05 was considered statistically significant.

Table 5. Allele frequencies of 4 *HMGB1* SNPs and their association with clinical status in patients with breast cancer.

Gene	HER2 + TNBC (N=188) n (%)														
Alle	Clinical Stage			Tumor size			Lymph node metastasis			Distant metastasis			Pathological grade		
	Stage I/II	Stage III/IV	OR (95% CI)	≦T2	> T2	OR (95% CI)	N0+N1	N2+N3	OR (95% CI)	M0	M1	OR (95% CI)	G1+G2	G3	OR (95% CI)
rs1360485															
A	107 (75.4)	31 (67.4)	1.00 (reference)	135 (95.1)	35 (76.1)	1.00 (reference)	69 (48.6)	19 (41.3)	1.00 (reference)	137 (96.5)	39 (84.8)	1.00 (reference)	57 (40.1)	19 (41.3)	1.00 (reference)
G	35 (24.6)	15 (32.6)	1.48 (0.72-3.06)	7 (4.9)	11 (23.9)	**6.06 (2.19-16.77)***	73 (51.4)	27 (58.7)	1.34 (0.69-2.63)	5 (3.5)	7 (15.2)	**4.92 (1.48-16.35)***	85 (59.9)	27 (58.7)	0.95 (0.49-1.87)
rs1045411															
G	109 (74.1)	29 (70.7)	1.00 (reference)	137 (93.2)	33 (80.5)	1.00 (reference)	73 (49.7)	15 (36.6)	1.00 (reference)	142 (96.6)	34 (82.9)	1.00 (reference)	58 (39.5)	18 (43.9)	1.00 (reference)
A	38 (25.9)	12 (29.3)	1.19 (0.55-2.56)	10 (6.8)	8 (19.5)	**3.32 (1.22-9.07)***	74 (50.3)	26 (63.4)	1.71 (0.84-3.49)	5 (3.4)	7 (17.1)	**5.85 (1.75-19.55)***	89 (60.5)	23 (56.1)	0.83 (0.41-1.68)
rs2249825															
C	115 (74.7)	23 (67.6)	1.00 (reference)	145 (94.2)	25 (73.5)	1.00 (reference)	77 (50.0)	11 (32.4)	1.00 (reference)	146 (94.8)	30 (88.2)	1.00 (reference)	61 (39.6)	15 (44.1)	1.00 (reference)
G	39 (25.3)	11 (32.4)	1.41 (0.63-3.16)	9 (5.8)	9 (26.5)	**5.80 (2.10-16.03)***	77 (50.0)	23 (67.6)	2.09 (0.95-4.58)	8 (5.2)	4 (11.8)	2.43 (0.69-8.60)	93 (60.4)	19 (17.0)	0.83 (0.39-1.76)
rs1412125															
T	105 (74.5)	33 (70.2)	1.00 (reference)	134 (95.0)	36 (76.6)	1.00 (reference)	62 (44.0)	26 (55.3)	1.00 (reference)	132 (93.6)	44 (93.6)	1.00 (reference)	63 (44.7)	13 (27.7)	1.00 (reference)
C	36 (25.5)	12 (29.8)	1.24 (0.6-2.57)	7 (5.0)	11 (23.4)	**5.85 (2.12-16.17)***	79 (56.0)	21 (44.7)	0.63 (0.33-1.23)	9 (6.4)	3 (6.4)	1.00 (0.26-3.86)	78 (55.3)	34 (72.3)	**2.11 (1.03-4.34)***

The odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were estimated using logistic regression models, age, tobacco and alcohol consumption.

SNP = single nucleotide polymorphism; HMGB1 = high-mobility group box protein 1; HER2 = human epidermal growth factor receptor 2; TNBC = triple-negative breast cancer; T2 = tumor >20 mm but ≤50 mm in greatest dimension; N0 = no regional lymph node metastasis; N1 = metastasis to movable ipsilateral level I, II axillary lymph node(s); N2 = metastases in ipsilateral level I, II axillary lymph nodes that are clinically fixed or matted or in clinically detected ipsilateral internal mammary nodes in the absence of clinically evident axillary lymph node metastasis; N3 = Metastases in ipsilateral infraclavicular (level III axillary) lymph node(s), with or without level I, II axillary node involvement, or in clinically detected ipsilateral internal mammary lymph node(s) and in the presence of clinically evident level I, II axillary lymph node metastasis; or metastasis in ipsilateral supraclavicular lymph node(s), with or without axillary or internal mammary lymph node involvement; M0 = no clinical or radiographic evidence of distant metastasis; M1 = distant detectable metastases as determined by classic clinical and radiographic means and/or histologically proven >0.2 mm; G1 = well differentiated; G2 = moderately differentiated; G3 = poorly differentiated.

* p value < 0.05 was considered statistically significant.

Breast cancer is the most commonly diagnosed neoplasm and the third leading cause of cancer-associated mortality in the United States, with 22.2 mortalities per 100,000 women associated with breast cancer each year [29]. The 5-year relative survival rate for breast cancer has gradually increased since the early 1990s; between 2007 and 2011 it was ~89.2% [29]. The prognosis of patients with breast cancer is critically dependent on the disease stage at the time of diagnosis. Therefore, it is important to increase screening rates and genetic testing for hereditary breast cancer, to increase the chances of early diagnosis [30, 31]. The current study is the first to examine the distribution of the rs1360485,

rs1045411, rs2249825 and rs1412125 SNPs and their possible association with breast cancer development. We also investigated the associations of these *HMGB1* SNPs with clinical status, clinical pathologic markers, and susceptibility for breast cancer. In analyses adjusted for potential confounding factors, there were no significant differences between cases and controls in regard to the frequency of rs1360485, rs1045411, rs2249825 and rs1412125 polymorphisms. However, the presence of one G allele in the rs1360485 SNP, one G allele in the rs2249825 SNP, or one C allele in the rs1412125 SNP increased the likelihood of developing T2 breast cancer. Moreover, having one G allele in the rs1360485 SNP, one A allele in the rs1045411 SNP, or one G allele in the rs2249825 SNP was associated with a higher likelihood of developing lymph node metastatic disease. These results indicate that *HMGB1* SNPs contribute to tumor size and lymph node metastasis in breast cancer patients.

This study found that having one G allele in the rs1360485 SNP or one G allele in the rs2249825 SNP increased the risk of developing T2 breast cancer and distant metastasis in HER2 and TNBC subtypes when compared with luminal A and luminal B subgroups. Similarly, having one G allele in the rs2249825 or one C allele in the rs1412125 increases the risk of developing T2 breast cancer in HER2 and TNBC breast cancer subtypes. It is already established that overexpression of the *HMGB1* gene is implicated in the development, invasion and metastasis of breast cancer [32]. In addition, HMGB1 is involved in the chemotherapeutic resistance of breast cancer cells [33, 34]. However, more research is required to determine whether an association exists among advanced-stage disease, HMGB1 expression levels and *HMGB1* genotype, and clarification is needed in regard to the effects of the *HMGB1* genotype on breast cancer risk.

In conclusion, our results demonstrate an association between *HMGB1* gene variants and the risk of breast cancer. However, we dose not recruited the survival results of breast cancer patients. Future research could evaluate the association of *HMGB1* polymorphisms with survival of breast cancer patients. We show that *HMGB1* gene variants significantly increase the risk of developing T2 breast cancer and lymph node metastasis among Chinese Han females. This study indicates a correlation exists between *HMGB1* polymorphisms and breast cancer risk. *HMGB1* may therefore serve as a predictive marker for breast cancer therapy.

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Competing Interests

The authors have declared that no competing interest exists.

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