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Genetic Variation in Metastasis-Associated in Colon Cancer-1 and the Risk of Breast Cancer Among the Chinese Han Population

A STROBE-Compliant Observational Study

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Abstract: Metastasis-associated in colon cancer-1 (MACC1), a newly identified oncogene, is involved in angiogenesis, invasiveness, and metastasis in many cancers. Epidemiological studies have indicated the associations between MACC1 polymorphisms and cancer risk. However, the association between genetic polymorphisms in MACC1 and breast cancer (BC) was not clear. This study aimed to evaluate the relationship between MACC1 polymorphisms and BC risk.

We genotyped 4 single-nucleotide polymorphisms (SNPs) in MACC1 (rs975263, rs1990172, rs3735615, rs4721888) to determine the haplotypes in 560 BC patients and 583 age-, sex-, and ethnicity-matched healthy individuals. Genotypes were determined using the Sequenom MassARRAY method. We estimated the odds ratios (ORs) and 95% confidence intervals (95% CIs) using the chi-square test.

There were significant differences between patients and controls in the MACC1 rs975263 allelic (T vs C: OR = 0.76, 95% CI = 0.61–0.95, $P = 0.014$) and genotypic groups (TC vs TT: OR = 0.70, 95% CI = 0.54–0.92, $P = 0.009$; TC+CC vs TT: OR = 0.71, 95% CI = 0.55–0.92, $P = 0.008$). Analysis of clinical features demonstrated significant associations between rs975263 and Scarff–Bloom–Richardson (SBR) grade 3 cancer ($P = 0.006$) and postmenopausal women ($P = 0.018$). Compared with the rs4721888 CC genotype, the frequency of rs4721888 GC and GC+CC variants was higher in patients. Further analysis revealed that the variant genotypes were positively associated with lymph node metastasis. However, we failed to find any relationships between rs1990172 or rs3735615 polymorphism and BC risk. In addition, haplotype analysis indicated that the CTGG and CTCG haplotypes (rs975263, rs1990172, rs3735615, rs4721888) were significantly associated with decreased susceptibility to BC ($P = 0.029$ and 0.019 respectively).

Our results suggest that rs975263 and rs4721888 polymorphisms in MACC1 are associated with the risk of BC susceptibility and may be involved in the progression of BC in Chinese women.

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Abbreviations: BC = breast cancer, BRCA1 = breast cancer susceptibility gene 1, CIs = confidence intervals, ER = estrogen receptor, HER = human epidermal growth factor receptor, HGF = Hepatocyte growth factor, HWE = Hardy–Weinberg equilibrium, LN = lymph node, MACC1 = metastasis-associated in colon cancer-1, ORs = odds ratios, PR = progesterone receptor, SNPs = single nucleotide polymorphisms, SRP = Scarff–Bloom–Richardson.

INTRODUCTION

Breast cancer (BC) is the most common cancer in women and its incidence has increased in recent years worldwide.[1] BC is a multifactorial disease caused by complex genetic and environmental factors.[2] Metastasis is the most important cause of deaths in breast cancer patients. Candidate genetic risk factors may alter BC onset and outcome may include allele variants in oncogenes.[3] Breast cancer susceptibility gene 1 (BRCA1) is a classic tumor suppressor gene involved in basic cellular functions, but its reduced expression increased risk of breast cancer development and associated with familial and sporadic breast cancer.[4] The newly identified gene, metastasis-associated in colon cancer 1 (MACC1), is suggested to be related with angiogenesis, invasiveness, and metastasis in many cancers.[5]

MACC1 gene is located on human chromosome 7 (7p21.1) and contains 7 exons and 6 introns. The coding cDNA contains 2559 nucleotides and is 1 of 21243 sequenced human cDNAs.[6] MACC1 was identified in a genome-wide analysis as a differentially expressed gene in primary tumors, metastases, and normal mucosa of subjects with colon cancer.[7] MACC1 has been suggested as an independent prognostic indicator of metastasis formation and metastasis-free survival for colon carcinoma patients.[8] Subjects with high MACC1 mRNA expression had a 5-year survival rate of 15% compared to 80% for those with low levels of MACC1 mRNA expression,

which indicated MACC1 mRNA expression may be prognostic for metastasis-free survival.[7] The expression of MACC1, a transcriptional activator, is significantly correlated with clinical staging and TNM classification of breast cancer.[9] Advanced hepatocellular carcinoma patients with higher expression of MACC1 mRNA and nuclear protein in tumorous tissues has a shorter post cryoablation median time to progression and overall survival than that with lower MACC1 expression. Therefore, distribution of MACC1 expression in hepatocellular carcinoma cells may help us to select the best appropriate patients for cryotherapy.[10] MACC1, as a key regulator of HGF-MET signaling, may be of therapeutic relevance.[11] MACC1 induces cell migration, invasion, proliferation, and regulates apoptosis in cancer cells.[12,13] In vivo, MACC1 causes tumor growth and metastasis.[14]

These features designate MACC1 as a gene that can be used to predict the risk of metastasis and guide further diagnostic and therapeutic decisions. Originally discovered in colon cancer, MACC1 overexpression has been demonstrated to promote tumor proliferation, invasion, and metastasis in a wide spectrum of solid tumors including colon cancer,[15] gastric carcinoma,[16] hepatocellular carcinoma,[17,18] osteosarcoma,[19] glioma,[20,21] lung,[22,23] esophageal,[24] pancreatic,[25] ovarian,[26,27] cervical cancer, and BC.[9,28]

Single nucleotide polymorphisms (SNPs) have been proposed to play an important role in genetic susceptibility to cancer. Numerous SNPs have been identified in the human MACC1 gene using sequence databases. Recently, some studies indicate rs975263, rs1990172 and rs3735615 in MACC1 are associated with clinical outcome for HER-2 positive breast cancer patients and rs975263 and rs1990172 showed a significant high risk of relapse in hepatocellular carcinoma patients after transplantation under the overdominant model.[29–31] The 4 MACC1 polymorphisms involved in this study (rs975263, rs1990172, rs3735615, and rs4721888) are annotated in NCBI databases. However, their association with breast cancer risk had not been studied before. To explore their occurrence and frequencies in BC and their association with tumor progression we conducted a case–control study to investigate the association of MACC1 polymorphisms and BC risk in the Chinese Han population.

METHODS

Study Subjects

The characteristics of the BC patients and cancer-free controls in our study are described in Table 1. BC patients (n = 560) were recruited between January 2013 and October 2014 at the Second Affiliated Hospital of Xi'an Jiaotong University, China.[32] Inclusion criteria were as follows: (a) patients were recruited without regard to age; (b) patients were pathologically confirmed to have sporadic BC; (c) patients who had another type of cancer were excluded from the study. Cancer-free controls (n = 583) were recruited from individuals seeking health care in the outpatient departments at the hospital and were frequency-matched to the cases by age (±5 years). All subjects were Han Chinese and residents of Northwest China. The study was approved by the institutional review board of Xi'an Jiaotong University (Xi'an, China). All of the participants were

TABLE 1. Distributions of Select Variables in Breast Cancer Cases and Cancer-Free Controls

Characteristics		Cases	Control	P Value*
Number		560	583	
Age (mean ± SD)		49.09 ± 11.02	48.80 ± 8.28	0.612
Menopausal status				
Premenopausal		264	281	
Postmenopausal		296	302	0.716
Procreative times				
<2		289	291	0.594
≥2		271	292	
Body mass index (kg/m²) (mean ± SD)		22.52 ± 2.84	22.95 ± 3.21	0.038
Tumor size	<2 cm	188		
	≥2 cm	372		
LN metastasis	Negative	236		
	Positive	324		
Histological grade	SBR 1–2	309		
	SBR 3	251		
Venous invasion	None–little	359		
	Moderate–severe	201		
ER	Negative	247		
	Positive	313		
PR	Negative	255		
	Positive	305		
Her-2	Negative	389		
	Positive	171		
Ki67	<50%	335		
	≥50%	225		

ER = estrogen receptor, LN = lymph node, PR = progesterone receptor, SD = standard deviation.
* T test or 2-sided x^2 test.
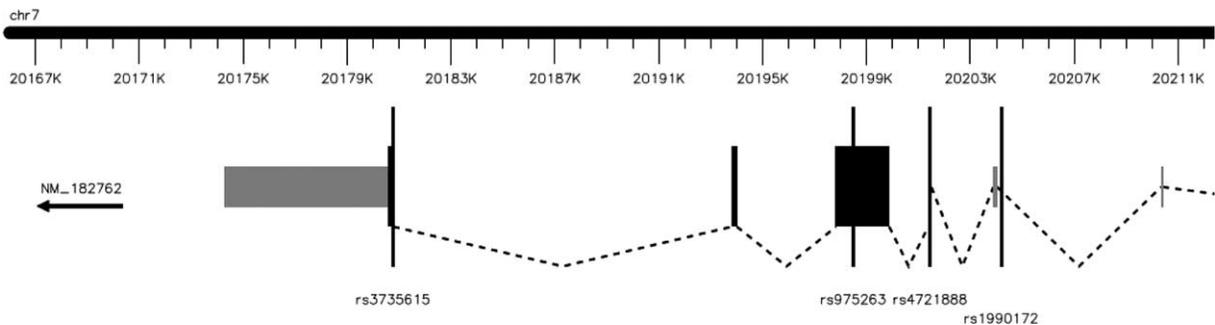
FIGURE 1. Genomic positions of selected MACC1 SNPs. MACC1 is located on the minus strand of chromosome 7. In the gene structure figure, the exons (coding exons) and untranslated regions (noncoding exons) were separately marked in black and gray, and the arrow under the gene label represented the strand direction. All selected SNPs are located in coding regions, except that rs1990172 is located in the intron region. MACC1 = metastasis-associated in colon cancer-1, SNPs = single nucleotide polymorphisms.

interviewed using a self-administered questionnaire includes a complete medical history, demographic data, and physical condition after obtaining written informed consent. We collected blood samples from all participants, and collected blood samples when the patients were pathologically confirmed to have BC before they received the chemotherapy or radiotherapy.

The cases and controls were well matched by age ($P = 0.612$). There was no significant difference in the distribution of menopausal state between the 2 groups ($P = 0.716$). However, the BMI (body mass index) was significantly different between BC patients and health controls ($P = 0.038$). The percentages of patients with tumors <2 cm and ≥2 cm in size were 33.6% and 66.4%, respectively. About 44.8% of the patients had Scarff–Bloom–Richardson (SBR) grade 3 cancer. The percentages of patients with lymph node involvement and venous invasion were 42.1% and 35.9%, respectively. In addition, the percentages of patients with estrogen receptor (ER)-, progesterone receptor (PR)-, human epidermal growth factor receptor 2 (HER2)-, and Ki67-positive disease were 55.9%, 54.5%, 30.5%, and 40.2%, respectively.

Genotyping Assay

Blood samples were collected in EDTA tubes and stored at −80 °C after centrifugation. DNA extraction carried out using the standard phenol–chloroform extraction method. DNA quantity was evaluated by spectrometry (DU530 UV/VIS spectrophotometer, Beckman Instruments, Fullerton, CA). Four tag SNPs (rs975263, rs1990172, rs3735615 and rs4721888) were selected for our study; these SNPs were with minor allele frequencies >5% in the HapMap Chinese Han Beijing (CHB) population (http://www.hapmap.org). All selected polymorphisms are located in coding regions with the exception of rs1990172, which is located in an intron (Figure 1). Sequenom MassARRAY Assay Design 3.0 Software (Sequenom, Inc, San Diego, CA) was used to design a Multiplexed SNP MassEX-TEND assay. We genotype the 4 polymorphisms in all subjects using a Sequenom MassARRAY RS1000 (Sequenom, Inc). Primers of PCR which were used for each SNP in our study are listed in Table 2. Sequenom Typer 3.0 Software (Sequenom, Inc) was used for data analyses.

Statistical Analyses

Allele and genotype frequencies of MACC1 polymorphisms were obtained by direct counts. SNP allele frequencies in the control subjects were tested for departure from Hardy–Weinberg Equilibrium (HWE) before analysis. HWE was evaluated by comparing expected and observed frequencies with algorithms in the Alrequin 3.1 program (L. Excoffier, CMPG, University of Bern, Switzerland). The statistical power of the case–control study was calculated using Power and Sample Size Calculation software (available on line: http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize). Differences between the cases and controls in the distributions of demographic characteristics, selected variables, and genotype frequencies of the 4 SNPs were evaluated using Student's t test or x^2 test. Associations between the genotypes of the MACC1 polymorphisms, the risk of BC and the patients' clinical characteristics were estimated by computing odds ratios (ORs) and 95% confidence intervals (CIs) from unconditional logistic regression analysis with an adjustment for age and body mass index. All of the statistical analyses were performed with the SPSS 18.0 software for Windows (PASW Statistics, SPSS Inc, Chicago, IL). We evaluated the risk in the dominant model (AA+ Aa vs aa; A represents the major allele, a the minor allele) and the recessive model (aa vs Aa+ AA) and the allele model (a vs A). A P value <0.05 was considered statistically significant, and all statistical tests were 2 sided.

RESULTS

Association Between MACC1 Polymorphisms and BC Risk

The genotype and allele frequencies of the MACC1 polymorphisms (rs975263, rs1990172, rs3735615, and rs4721888) are shown in Table 3. All polymorphisms conformed to HWE (rs975263: $P = 0.579$, rs1990172: $P = 0.757$, rs3735615: $P = 0.406$, and rs4721888: $P = 0.485$).

Compared with the TT genotype, the TC and TC+CC frequencies of rs975263 polymorphism among cases were significantly different from the controls (TC vs TT: OR = 0.70, 95% CI = 0.54–0.92, $P = 0.009$; TC+CC vs TT: OR = 0.71, 95% CI = 0.55–0.92, $P = 0.008$). The difference in the frequency distributions of T and C alleles among cases and controls was also significant (OR = 0.76, 95% CI = 0.61–0.95, $P = 0.014$). These results suggested that the MACC1 rs975263 polymorphism had a protective effect on BC risk. Compared with individuals with the rs4721888 GG genotype, individuals with GC and GC+CC genotypes had a significantly increased BC risk (GC vs GG: OR = 1.31, 95% CI = 1.03–1.67, $P = 0.029$; GC+CC vs GG: OR = 1.32, 95% CI = 1.04–1.67, $P = 0.020$). In addition, the minor allele C conferred an increased risk of BC in an allele model (C vs G: OR = 1.24, 95%CI = 1.03–1.51, $P = 0.027$). However, we did not observe

TABLE 2. Primers Used for this Study

SNP_ID	1st-PCRP	2nd-PCRP	UEP_SEQ
rs975263	ACGTTGGATGGAGCAGACTTGATTTCCTCC	ACGTTGGATGTCACTACTCCTGATCCAACC	cACCCCAAACCTAAAAAGACTCT
rs1990172	ACGTTGGATGGAAAAGGAGGGAAGCATGTG	ACGTTGGATGAGCATGCCACCTTATGAGAC	accCCTTATGAGACAATTTTTGGAT
rs3735615	ACGTTGGATGGATGTGGAAACCTGCCTATG	ACGTTGGATGTGTCCAAAGCTGACTGAAGG	cctcCTGAAGGTCTTGTAACACATCT
rs4721888	ACGTTGGATGTCGGTCAGGAAGAATTGCAC	ACGTTGGATGTCCCATACCTGTAATATTGC	gaCTGTAATATTGCAACTTTTTGAGA

significant association between the MACC1 rs1990172 or rs3735615 polymorphisms and BC risk in any genetic model, as shown in Table 3. We also obtained the statistical power of 0.89 and 0.80 for the 2 significant polymorphisms identified, rs975263 and rs4721888, respectively. This showed that our sample size of 1143 was adequate and the study was sufficiently able to detect the true association of these 2 polymorphisms with BC.

Stratified Analysis of MACC1 Polymorphisms and BC Risk

Stratified analysis of the effect of rs975263 and rs4721888 polymorphisms on BC by menopausal status is displayed in Table 4. The results indicated that rs975263 was associated with an increased BC susceptibility in postmenopausal women (OR = 1.53, 95% CI = 1.07–2.19, $P = 0.018$). However, there was no association between rs4721888 and BC risk in either premenopausal patients or postmenopausal patients.

Association Between MACC1 Polymorphisms and Clinical Parameters of BC Patients

MACC1 gene polymorphisms were also analyzed to establish their associations with clinicopathological features, including tumor size, lymph node metastasis, histological grade, venous invasion and the statuses of ER, PR, HER-2, and Ki67. The positive results are shown in Table 5. For rs975263, compared with the TT genotype, the TC+CC genotype appeared at lower frequencies in SBR grade 3 cases

TABLE 3. Genotype and Allele Frequencies of MACC1 Polymorphisms Among the Cases and Controls and the Associations With Breast Cancer Risk

SNP	Genotype	Control (583)	Case (560)	OR (95% CI)	*P*
rs975263 HWE = 0.579	T/T	385 (66.0%)	410 (73.2%)	1.00	
	T/C	175 (30.0%)	131 (23.4%)	0.70 (0.54–0.92)	0.009
	C/C	23 (4.0%)	19 (3.4%)	0.78 (0.42–1.45)	0.423
	T/C-C/C	198 (34.0%)	150 (26.8%)	0.71 (0.55–0.92)	0.008
	T	945 (81.0%)	951 (84.9%)	1.00	
	C	221 (19.0%)	169 (15.1%)	0.76 (0.61–0.95)	0.014
rs1990172 HWE = 0.757	G/G	412 (70.7%)	400 (71.4%)	1.00	
	G/T	155 (26.6%)	142 (25.4%)	0.94 (0.72–1.23)	0.669
	T/T	16 (2.7%)	18 (3.2%)	1.15 (0.58–2.30)	0.674
	G/T-T/T	171 (29.3%)	160 (28.6%)	0.96 (0.75–1.25)	0.777
	G	979 (84.0%)	942 (84.1%)	1.00	
	T	187 (16.0%)	178 (15.9%)	0.99 (0.79–1.24)	0.925
rs3735615 HWE = 0.406	G/G	410 (70.3%)	387 (69.1%)	1.00	
	G/C	161 (27.6%)	156 (27.9%)	1.02 (0.79–1.33)	0.844
	C/C	12 (2.1%)	17 (3.0%)	1.50 (0.71–3.18)	0.287
	G/C-C/C	173 (29.7%)	173 (30.9%)	1.06 (0.82–1.36)	0.654
	G	982 (84.1%)	930 (83.0%)	1.00	
	C	185 (15.9%)	190 (17.0%)	1.08 (0.87–1.35)	0.473
rs4721888 HWE = 0.485	G/G	353 (60.5%)	301 (53.7%)	1.00	
	G/C	205 (35.2%)	229 (40.9%)	1.31 (1.03–1.67)	0.029
	C/C	25 (4.3%)	30 (5.4%)	1.41 (0.81–2.45)	0.224
	G/C-C/C	230 (39.5%)	259 (46.3%)	1.32 (1.04–1.67)	0.020
	G	911 (78.1%)	831 (74.2%)	1.00	
	C	255 (21.9%)	289 (25.8%)	1.24 (1.03–1.51)	0.027

CI = confidence interval, HWE = Hardy–Weinberg Equilibrium, MACC1 = metastasis-associated in colon cancer-1, OR = odds ratio, SNP = single nucleotide polymorphism.
[*]Two-sided x^2 test for the distributions of genotype and allele frequencies.

TABLE 4. Stratification Analyses by Menopause Status Between MACC1 Polymorphisms and Risk of Breast Cancer

	rs975263					rs4721888			
Genotypes	Case (N = 560) N (%)	Control (N = 583) N (%)	P^*	OR (95%CI)	Genotypes	Case (N = 560) N (%)	Control (N = 583) N (%)	P^*	OR (95%CI)†
Pre-menopause					Pre-menopause				
TC+CC	70 (28.0%)	98 (34.6%)		1.00 (reference)	GC+CC	139 (48.1%)	115 (40.2%)		1.00 (reference)
TT	180 (72.0%)	185 (65.4%)	0.100	1.36 (0.94–1.97)	GG	150 (51.9%)	171 (59.8%)	0.057	0.73 (0.52–1.01)
Post-menopause					Post-menopause				
TC+CC	75 (24.6%)	100 (33.3%)		1.00 (reference)	GC+CC	120 (44.3%)	115 (38.7%)		1.00 (reference)
TT	230 (75.4%)	200 (66.7%)	0.018	1.53 (1.07–2.19)	GG	151 (55.7%)	182 (61.3%)	0.179	0.80 (0.57–1.11)

CI = confidence interval, MACC1 = metastasis-associated in colon cancer-1, OR = odds ratio.
* Two-sided x^2 test for the distributions of genotype frequencies.
† Adjusted for age and age at menarche.

(OR = 0.59, 95% CI = 0.40–0.86, *P* = 0.006). For rs3735615, we found that the Ki67 value of patients with the GC+CC genotype was more likely to be <50% compared with GG genotype carriers (OR = 0.66, 95% CI = 0.46–0.97, *P* = 0.03). Moreover, compared with the GG genotype, the GC+CC genotype of rs4721888 had a higher frequency in lymph node involvement (OR = 1.48, 95% CI = 1.05–2.07, *P* = 0.02). However, no statistical association was detected between the 4 variants and tumor size, venous invasion or the values of ER, PR, and HER-2.

Association of MACC1 Haplotypes With BC Risk

The relationship of MACC1 haplotypes with the risk of developing BC was also evaluated. The frequency distributions of 4 common MACC1 rs975263, rs1990172, rs3735615, and rs4721888 haplotypes are shown in Table 6, with the most frequent haplotype in the controls being chosen as the reference. Haplotype analysis indicated that the frequencies of CTGG and CTCG haplotypes (rs975263, rs1990172, rs3735615, rs4721888) were lower in patients than in controls (1.9% vs 3.6% and 1.4% vs 3.1%, respectively). Compared with the TGGG wild type, carriers of CTGG and CTCG haplotypes had significant associations with decreased susceptibility to BC (CTGG: OR = 0.55, 95% CI = 0.32–0.95, *P* = 0.029; CTCG: OR = 0.49, 95% CI = 0.27–0.90, *P* = 0.019).

DISCUSSION

Epidemiologic studies have suggested that single nucleotide polymorphisms (SNP) in MACC1 may contribute to individuals' susceptibility to cancer. Carriers of the G allele of rs1990172 showed a significantly decreased overall survival in colorectal cancer (additive hazard ratios = 1.38, 95%CI = 1.05–1.82, *P* = 0.023). Multivariate analysis adjusted for age and UICC tumor stage confirmed this result (hazard ratios = 1.49, 95% CI = 1.12–1.98, *P* = 0.007).[33] Other investigated genetic variants (rs3114446, rs10275612, rs3095007, rs3095009, and rs7780032) of the MACC1 gene were not significantly associated with overall survival.[33] For the rs975263 polymorphism, younger colon cancer individuals with CT genotype has a reduced survival with stage I or II.[29] Zheng et al[31] suggested that SNP rs1990172 and SNP rs975263 in MACC1 may be potential genetic markers for hepatocellular carcinoma recurrence in liver transplantation patients.

Muendlein et al[30] provided the first evidence that MACC1 polymorphisms are associated with clinical outcomes for HER2-positive BC patients.

In our study, we estimated the relationship between rs975263, rs1990172, rs3735615, and rs4721888 in MACC1 and BC susceptibility. Rs1990172 is located in the intronic region of the MACC1 gene and has no impact on coding exon of MACC1 gene.[30] Rs975263, rs3735615, and rs4721888 are all missense alterations. Rs975263, which is situated at codon 515, fifth exon, has a nonsynonymous substitution of leucine to serine which may lead to a loss of phosphorylation site.[31] Rs3735615 results in a substitution of threonine for arginine that potentially can act as phosphorylation site. Rs4721888 exchanges the amino acid sequence from leucine to valine, which has an unsure effect because leucine and valine are all nonpolar amino acids. Schmid et al[29] found rs975263 and rs4721888 variants are possibly benign for they were not in predicted domains of MACC1 structure, whereas rs3735615 variant could be damaging for it lies in a conserved domain.

Overall, we observed that variant genotypes of MACC1 rs975263 and rs4721888, but not rs1990172 or 3735615, were associated with BC risk. Rs975263 was associated with protection from BC, but rs4721888 increased BC susceptibility. The results are partially consistent with other studies. For example, Muendlein et al[30] found that carriers of the rs975263 T allele had an adverse effect on cancer prognosis, the rs1990172 G allele was associated with an increased risk of cancer progression or death and the rs3735615 C allele had a protective impact on overall survival.[30] We also observed that the variant rs975263 genotypes in the MACC1 gene were inversely associated with SBR grade 3 and that rs4721888 was related to positive lymph node metastasis. Furthermore, the variant genotypes of rs975263 were more frequent in postmenopausal women. The results suggest that rs975263 had a protect effect on BC patients and rs47218888 polymorphism in MACC1 is related to the development and progression of BC and may help to accurately predict the clinical course of BC. In addition, compared with the TGGG wild type, the CTGG and CTCG haplotypes were significantly associated with decreased susceptibility to BC (*P* = 0.029 and *P* = 0.019, respectively).

Our study had some limitations. First, the sample size was inadequate for a stratified analysis and for an analysis of these associations in patients with BC. Second, we did not investigate whether predisposing factors, including high-dose radiation

TABLE 5. The Associations Between MACC1 Polymorphisms and Clinical Characteristics of Breast Cancer Patients

Variables	rs975263					rs1990172					rs3735615					rs4721888				
	TT	TC+CC	P^*	OR†	95% CI	GG	GT+TT	P^*	OR†	95% CI	GG	GC+CC	P^*	OR†	95% CI	GG	GC+CC	P^*	OR†	95% CI
Tumor size	410	150				400	160				387	173				301	259			
<2 cm 188	145	43		1.00	(reference)	129	59		1.00	(reference)	123	65		1.00	(reference)	109	79		1.00	(reference)
≥2 cm 372	265	107	0.14	1.36	(0.91–2.05)	271	101	0.30	0.82	(0.56–1.20)	264	108	0.18	0.77	(0.53–1.13)	192	180	0.15	1.29	(0.91–1.84)
LN metastasis																				
Negative 236	177	59		1.00	(reference)	176	60		1.00	(reference)	173	63		1.00	(reference)	140	96		1.00	(reference)
Positive 324	233	91	0.42	1.17	(0.80–1.72)	231	93	0.39	1.18	(0.81–1.73)	214	110	0.07	1.41	(0.98–2.04)	161	163	0.02	1.48	(1.05–2.07)
Histological grade																				
SBR 1–2 309	212	97		1.00	(reference)	220	89		1.00	(reference)	213	96		1.00	(reference)	169	140		1.00	(reference)
SBR 3 251	198	53	0.006	0.59	(0.40–0.86)	180	71	0.89	0.98	(0.67–1.41)	174	77	0.92	0.98	(0.69–1.41)	132	119	0.62	1.09	(0.78–1.52)
Venous invasion																				
None-little 359	270	89		1.00	(reference)	261	98		1.00	(reference)	258	101		1.00	(reference)	191	168		1.00	(reference)
Moderate-severe 201	140	61	0.15	1.32	(0.90–1.94)	139	62	0.37	1.19	(0.81–1.74)	129	72	0.06	1.43	(0.99–2.06)	110	91	0.73	0.94	(0.67–1.33)
ER																				
Negative 247	175	72		1.00	(reference)	171	76		1.00	(reference)	164	83		1.00	(reference)	136	111		1.00	(reference)
Positive 313	235	78	0.26	0.81	(0.55–1.17)	229	84	0.31	0.83	(0.57–1.19)	223	90	0.22	0.80	(0.56–1.14)	165	148	0.58	1.09	(0.79–1.54)
PR																				
Negative 255	179	76		1.00	(reference)	184	71		1.00	(reference)	168	87		1.00	(reference)	138	117		1.00	(reference)
Positive 305	231	74	0.14	0.75	(0.52–1.10)	216	89	0.73	1.07	(0.74–1.54)	219	86	0.13	0.76	(0.53–1.09)	163	142	0.87	1.03	(0.74–1.43)
HER-2																				
Negative 389	298	91		1.00	(reference)	279	110		1.00	(reference)	275	114		1.00	(reference)	213	176		1.00	(reference)
Positive 171	122	49	0.19	1.32	(0.88–1.97)	121	50	0.81	1.05	(0.71–1.56)	112	59	0.22	1.27	(0.87–1.87)	88	83	0.47	1.14	(0.80–1.64)
Ki67																				
<50% 335	248	87		1.00	(reference)	247	88		1.00	(reference)	220	115		1.00	(reference)	187	158		1.00	(reference)
≥50% 225	162	63	0.59	1.11	(0.76–1.62)	153	72	0.14	1.32	(0.91–1.91)	167	58	0.03	0.66	(0.46–0.97)	114	101	0.79	1.05	(0.75–1.48)

CI = confidence interval, ER = Estrogen receptor, HER-2 = human epidermal growth factor receptor 2, LN = axillary lymph node, OR = odds ratio, PR = progesterone receptor, SBR = (Scarff, Bloom, and Richardson) tumor grade (1–2 vs 3).
* Two-sided x^2 test for the distributions of genotype frequencies.
† Adjusted for tumor size, lymph node involvement, histological grade, venous invasion, ER, PR, HER-2, and Ki67 status.

TABLE 6. The Haplotype Frequencies of MACC1 Polymorphisms and Breast Cancer Risk

Haplotype rs975263	rs1990172	rs3735615	rs4721888	Controls (N = 1166) n, %	Patients (N = 1120) n, %	OR (95% CI)	*P*
T	G	G	G	455 (39.0%)	412 (36.8%)	1.00 (reference)	
T	G	G	C	176 (15.1%)	195 (17.4%)	1.22 (0.96–1.56)	0.104
T	G	C	G	143 (12.3%)	159 (14.2%)	1.23 (0.95–1.60)	0.125
C	G	G	G	127 (10.9%)	111 (9.9%)	0.96 (0.72–1.29)	0.809
T	T	G	G	103 (8.8%)	108 (9.6%)	1.15 (0.86–1.57)	0.339
C	G	G	C	56 (4.8%)	68 (6.1%)	1.34 (0.92–1.96)	0.127
C	T	G	G	42 (3.6%)	21 (1.9%)	0.55 (0.32–0.95)	0.029
C	T	C	G	36 (3.1%)	16 (1.4%)	0.49 (0.27–0.90)	0.019
Others				28 (2.4%)	30 (2.7%)	1.18 (0.70–2.01)	0.535

CI = confidence interval, MACC1 = metastasis-associated in colon cancer-1, OR = odds ratio.

exposure, alcohol consumption, and postmenopausal obesity, were associated with the risk of BC because of a lack of such data from both patients with BC and controls. The effect of these factors on BC risk should be assessed in a future study.

In summary, our case–control study indicates that rs975263 and rs4721888 in MACC1 have significant effects on the susceptibility and progression of BC among Chinese women. Further functional studies and larger population-based prospective studies are required to further elucidate the impact of MACC1 polymorphisms on BC.

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