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MMP9 Polymorphisms and Breast Cancer Risk: A Report from the Shanghai BreastCancer Genetics Study

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Abstract

Purpose—In addition to tumor invasion and angiogenesis, matrix metalloproteinase (MMP)9 also contributes to carcinogenesis and tumor growth. Genetic variation that may influence MMP9 expression was evaluated among participants of the Shanghai Breast Cancer Genetics Study (SBCGS) for associations with breast cancer susceptibility.

Methods—In stage 1, 11 *MMP9* SNPs were genotyped by the Affymetrix Targeted Genotyping System and/or the Affymetrix Genome-Wide Human SNP Array 6.0 among 4,227 SBCGS participants. One SNP was further genotyped using the Sequenom iPLEX MassARRAY platform among an additional 6,270 SBCGS participants. Associations with breast cancer risk were evaluated by odds ratios (OR) and 95% confidence intervals (CI) from logistic regression models that included adjustment for age, education, and genotyping stage when appropriate.

Results—In Stage 1, rare allele homozygotes for a promoter SNP (*rs3918241*) or a non-synonymous SNP (*rs2274756*, *R668Q*) tended to occur more frequently among breast cancer cases (*p*-value = 0.116 and 0.056, respectively). Given their high linkage disequilibrium ($D' = 1.0$, $r^2 = 0.97$), one (*rs3918241*) was selected for additional analysis. An association with breast cancer risk was not supported by additional Stage 2 genotyping. In combined analysis, no elevated risk of breast cancer among homozygotes was found (OR: 1.2, 95% CI: 0.8–1.8).

Conclusions—Common genetic variation in *MMP9* was not found to be significantly associated with altered breast cancer susceptibility among participants of the Shanghai Breast Cancer Genetics Study.

Keywords

matrix metalloproteinase 9; polymorphisms; breast cancer; susceptibility

Introduction

Known predominantly as a mediator of tumor invasion and angiogenesis [1–3], matrix metalloproteinase (MMP)9 (aka 92-kD type IV collagenase or gelatinase B) has also been shown to contribute to carcinogenesis and tumor growth. Laboratory experiments have demonstrated that the transformation of mammary epithelial cells requires MMP9 [4] and

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that the tumorigenic potential of cultured cells correlated with their MMP9 expression [5]. In human samples, total MMP9 activity was significantly higher in malignant breast cancers than in benign breast tumors [6], and circulating MMP9 levels from breast cancer patients were significantly higher than serum levels in women with benign breast disease or healthy controls [7,8]. In fact, reports of MMPs in breast cancer tissue and serum samples have been consistently reported to be increased, and so a recent review called for continued and expanded evaluation of MMPs and hormone-related cancers [9].

Genetic variation that influences MMP9 expression may contribute to cancer susceptibility. Numerous *MMP9* polymorphisms have been reported, of which several are considered to be functional. Two promoter polymorphisms have been shown to influence expression, including a multi-allelic dinucleotide repeat (CA)_n (*rs3222264*) that has lower transcription from a 14 repeat allele than from other alleles [10,11], and a C to T substitution at -1562 (*rs3918242*) that results in the loss of a putative transcriptional repressor binding site and a subsequent increase in *MMP9* transcription [12]. Non-synonymous coding SNPs in *MMP9* include *rs1805088* (A20V) in exon 1, *rs17576* (R279Q) in exon 6, *rs2250889* (P574R) in exon 10, and *rs2274756* (R668Q) in exon 12.

In relation to cancer risk, the -1562 C/T SNP (*rs3918242*) has been most frequently evaluated, although results have been inconsistent. The C allele has been associated with a decreased risk of prostate cancer [13], and increased risks of squamous cell carcinoma of the lung [14], endometrial carcinoma [15], and colorectal cancer [16]. However, another study of prostate cancer found no association [17], as did two other studies for colorectal cancer [18,19], and one study of ovarian cancer [20]. In regards to breast cancer, two smaller studies found no association [21,22], while one larger study found a moderately increased risk among T allele homozygotes [23]. Other *MMP9* polymorphisms have also been evaluated for altered cancer susceptibility. One study evaluated the dinucleotide repeat (CA)_n (*rs3222264*) and colorectal cancer, and found no association [16]. Four studies genotyped the non-synonymous SNP *rs17576* G/A (R279Q) and had mixed associations with cancer risk. A allele homozygotes had a non-significantly increased risk of lung cancer [24], but no association with colorectal cancer [16]. For prostate cancer, A allele homozygotes had an increased risk in one small study [25], but no association in another large study [17]. Another non-synonymous SNP *rs2274756* (R668Q) was evaluated in two studies; no association was found for either lung cancer [24] or prostate cancer [17]. To the best of our knowledge, no study has yet evaluated any of these non-synonymous *MMP9* SNPs in relation to breast cancer risk. In the most comprehensive study to date, Jacobs et al. evaluated two intronic SNPs (*rs3918256* and *rs3787268*) in addition to the three previously described SNPs (*rs17576*, *rs2250889*, and *rs2274756*) among 1,425 cases and 1,453 controls, and found no associations with altered prostate cancer susceptibility [17]; no comparable study of breast cancer risk has yet been reported.

Thus, this study was undertaken to comprehensively assess individual genetic variation across the *MMP9* gene, and evaluate associations with breast cancer susceptibility among participants of the Shanghai Breast Cancer Genetics Study (SBCGS).

Methods

Subjects were participants of three population based studies conducted among Chinese women in urban Shanghai: the Shanghai Breast Cancer Study (SBCS), the Shanghai Breast Cancer Survival Study (SBCSS), and The Shanghai Endometrial Cancer Study (SECS). Collectively, these studies comprise the Shanghai Breast Cancer Genetics Study (SBCGS); detailed methodologies for these studies have been previously reported [26]. Briefly, the SBCS is a large two-stage, population-based, case-control study. Breast cancer cases were

identified via a rapid case-ascertainment system and the Shanghai Cancer Registry; diagnoses were confirmed by two senior pathologists. Controls were randomly selected using the Shanghai Resident Registry. SBCS I recruitment occurred between August 1996 and March 1998, and included women aged 25–65. SBCS II recruitment occurred from April 2002 to February 2005, and was expanded to include women aged 20–70. Also included in the study were cases recruited between April 2002 and December 2006 as part of the SBCSS, a population-based study of newly diagnosed breast cancer cases identified by the Shanghai Cancer Registry, and controls recruited between January 1997 and December 2003 as part of the SECS, a population-based case-control study of endometrial cancer that included women aged 25–70 that was conducted in a manner almost identical to the SBCS. Of those eligible, 1,459 cases (91.1%) and 1,556 controls (90.3%) from SBCS I, 1,989 cases (83.7%) and 1,989 (70.4%) controls from SBCS II, and 5,046 cases (80.1%) from SBCSS and 1,212 controls (74.4%) from SECS completed in-person interviews with structured questionnaires. Blood or buccal cell samples were donated and available for 1,193 cases (81.8%) and 1,310 controls (84.2%) from SBCS I, 1,932 cases (97.1%) and 1,857 controls (93.4%) from SBCS II, and 4,845 (96.0%) cases from SBCSS and 1,039 (85.7%) controls from SECS. Because of a time overlap in subject recruitment, 1,469 breast cancer patients participated in both the SBCS II and the SBCSS, and 109 controls participated in both the SBCS I and SECS, so that the actual number of participants included in the current analysis from SBCSS and SECS was 3,466 and 930, respectively. Genomic DNA was extracted using commercial DNA purification kits. Informed consent was granted by all included participants. Approval was granted from relevant review boards in both China and the United States.

Stage 1 genotyping included haplotype tagging SNPs (htSNPs) and SNPs included on the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix). htSNPs were selected using Han Chinese data presented in the HapMap Project [27] using the Tagger program [28] to capture SNPs with a minimum minor allele frequency (MAF) of 0.05 in the *MMP-9* gene ($\pm$ 5kb) with an r^2 of 0.90 or greater. Twelve *MMP-9* SNPs were selected, but assay design failed for three SNPs (*rs3918242*, *rs2236416*, and *rs3787268*), and genotyping did not pass quality control for three SNPs (*rs3848722*, *rs6065913*, and *rs3918254*), so that six SNPs were successfully designed and genotyped for 1,062 cases and 1,069 controls from SBCS I, using a Targeted Genotyping System (Affymetrix, Santa Clara, CA). To increase the density of genetic markers in this study, data from our Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix) was included for five additional *MMP-9* polymorphisms ($\pm$ 10kb) that were genotyped among 1,104 cases and 1,109 controls from SBCS I, and 969 cases and 975 controls from SBCS II. Additional genotyping was performed for one promising SNP from Stage 1 (*rs3918241*) and one SNP reported in the literature to be functional (*rs3918242*) [12] with the Sequenom iPLEX MassARRAY platform (Sequenom) among 3,453 SBCSS cases and 914 SECS controls, as well as 11 cases and 126 controls from SBCS I and 961 cases and 875 controls from SBCS II that were not previously genotyped. For *rs3918241*, Stage 2 genotyping included participants genotyped by the Sequenom platform. For *rs3918242*, similar to Affymetrix methods, Sequenom genotyping also failed.

Hardy-Weinberg equilibrium (HWE) was tested by comparing observed and expected genotype frequencies among controls (χ^2 -test). Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were determined by logistic regression analyses using models that included adjustment for age, education, and genotyping stage when appropriate. Linkage disequilibrium (LD) was assessed by Haploview [29]. Haplotype analysis was conducted with Hapstat [30]. Statistical significance was determined with by a p-value $\leq$ 0.05; all statistical tests were two-tailed.

Results

A total of 10,497 women were included in the current study; 4,227 were genotyped in Stage 1 and 6,270 were genotyped in Stage 2 (Table 1). Women in the two genotyping stages were generally comparable. As expected, breast cancer cases were found to differ from controls in regards to known breast cancer risk factors; cases were more likely to have earlier age at menarche, older age at first live birth, a history of breast fibroadenomas, a history of breast cancer among a first degree relative, a higher body mass index (BMI) and/or waist-to-hip ratio (WHR), and less likely to participate in regular physical activity than controls.

A total of 11 *MMP9* SNPs were included in the current study; of these, four were found to have minor allele frequencies (MAFs) of less than 5% (*rs11696804*, *rs6104416*, *rs13040272*, and *rs11086991*). None of the remaining seven polymorphisms were found to deviate from HWE. Estimates of effect on breast cancer risk, adjusted for age, education, and genotyping stage (when appropriate) are shown in Table 2. Two *MMP9* SNPs were found to have associations of marginal significance with breast cancer risk in recessive models (data not shown). Rare allele homozygotes for the promoter SNP *rs3918241* and the non-synonymous exon 12 SNP *rs2274756* both tended to be more likely to be breast cancer cases. These two polymorphisms were found to be in high LD ($D' = 1.0$, $r^2 = 0.97$) (Figure 1). As *rs3918241* had the larger MAF and was located in the promoter of the gene, it was selected for additional genotyping. Results from Stage 1 genotyping were not supported in Stage 2; no association was seen for *rs3918241* and breast cancer risk among 4,419 cases and 1,851 controls (Table 2). All seven of the *MMP9* SNPs genotyped were found to be in moderately high LD ($D' = 0.96$), and to be in one haplotype block (Figure 1). Results from haplotype analysis were generally consistent with results from single SNP analysis; there were no significant associations with breast cancer risk (data not shown).

Discussion

Common genetic variation across *MMP9* was systematically evaluated among participants of the Shanghai Breast Cancer Genetics Study. Two SNPs in high LD had suggestive associations with breast cancer risk in Stage 1, however, additional genotyping and analysis did not support a significant association.

Several functional polymorphisms in *MMP9* have been reported and evaluated for associations with cancer risk, including -1562 C/T (*rs3918242*) [13–16,18–23]. In a small study of French Caucasians, the C allele was associated with more than three-fold increased risk of squamous cell carcinoma of the lung [14]. However, in a small study among Tunisian men, the T allele was associated with an almost three-fold increased risk of developing prostate cancer [13]. Moderately sized studies of *MMP9* -1562 C/T (*rs3918242*) have also had mixed results. While no association was found for ovarian cancer among Chinese women [20], the C allele was significantly more frequent among Chinese endometrial carcinoma cases than controls [15]. Two studies of colorectal cancer found no association in Swedish [18] or Chinese populations [19], while a third found that Korean C allele homozygotes had an increased risk of colorectal cancer compared to T allele carriers [16]. Studies on breast cancer risk and *MMP9* -1562 C/T (*rs3918242*) have also had mixed results with both a moderate association [23] and null findings reported [21,22]. Other *MMP9* functional polymorphisms include a dinucleotide repeat (CA)_n (*rs3222264*), which was not found to be associated with colorectal cancer [16], and several non-synonymous SNPs, including *rs2274756* (*R668Q*) which was not associated with lung [24] or prostate cancer [17], and *rs17576* G/A (*R279Q*) which was not associated with lung cancer [24], colorectal cancer [16] or consistently associated with prostate cancer [17,25].

In the current study, three non-synonymous SNPs in *MMP9* were evaluated, including *rs17576* (*R279Q*) in exon 6, *rs2250889* (*P574R*) in exon 10, and *rs2274756* (*R668Q*) in exon 12. Of these, *rs2274756* (*R668Q*) minor allele homozygotes had a non-significantly increased risk of breast cancer in Stage 1. This SNP was found to be in LD with the promoter SNP *rs3918241*, which was further genotyped in Stage 2, where an association with breast cancer risk was not supported. The functional promoter SNP -1562 C/T (*rs3918242*) was not genotyped in the current study, as assay design by both Affymetrix and Sequenom Targeting genotyping methods were unsuccessful, possibly due to a higher than average GC content surrounding this polymorphism. Further, as *rs3918242* has not been genotyped among HapMap samples, LD between it and our SNPs can not be directly assessed. However, several lines of evidence indicate that its variation may have been captured in the current study. First, an early study reported that the -1562 C/T SNP (*rs3918242*) was in very high LD (p-value < 10^{-6}) with the *R279Q* SNP (*rs17576*) [12], and in our data, all *MMP9* SNPs were found to be in one LD block, with the smallest *D'* value between SNPs of 0.96. Second, the functional promoter SNP *rs3918242* is located only 240 base pairs away from *rs3918241*, and has a similar MAF of approximately 14% among controls in other large studies [23,31]. Because of the close proximity of these two variants, as well as the high LD of SNPs across the *MMP9* gene, we believe that *rs3918242* should be captured reasonably well in our study by *rs3918241* and other *MMP9* SNPs. Further, the recessive association with breast cancer risk seen in Stage 1 genotyping for *rs3918241* (OR: 1.7, 95% CI: 0.9–3.5) is similar to that reported for the rare allele by Lei et al (OR: 1.9, 95% CI: 1.0–3.6) for *rs3918242* [23]. However, in our study, additional genotyping did not support the suggested association.

Strengths of the current study include a large population, a two stage genotyping design to minimize type I error, and good coverage of the genetic variation in the *MMP9* gene. The seven genotyped SNPs with MAFs > 0.05 are estimated to cover approximately 83% of SNPs in HapMap with a MAF > 5% in the *MMP9* gene (± 10 kb) with an r^2 of 0.8. Limitations include the failure to directly evaluate the functional promoter SNP *rs3918242* (-1562 C/T) and the dinucleotide repeat (CA)_n (*rs3222264*), although the high LD across the *MMP9* gene suggests that these variants may be captured by our genotyping. We evaluated a total of 11 *MMP9* polymorphisms, including three non-synonymous SNPs. No variants were found to be significantly associated with breast cancer risk, indicating that any resulting differences in the *MMP9* protein or its expression are insufficient to culminate into measurable differences in breast cancer risk. In summary, common genetic variation in *MMP9* was not found to influence breast cancer susceptibility among participants of the Shanghai Breast Cancer Genetics Study.

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Abbreviations

LD	linkage disequilibrium
MMP	matrix metalloproteinase
SNP	single nucleotide polymorphism

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Figure 1. Linkage Disequilibrium of MMP9 Polymorphisms Among 2,148 Controls, The Shanghai Breast Cancer Genetics Study
Value shown is r^2 , all D' values were 0.96 or greater.

Table 1
Characteristics of Shanghai Breast Cancer Genetics Study Participants, by Genotyping Stage

Characteristics	Stage 1 (4,227)			Stage 2 (N=6,270)		
	Cases (N=2,079)	Controls (N=2,148)	p-value	Cases (N=4,419)	Controls (N=1,851)	p-value
Demographic Factors						
Age (years)	49.3 ± 8.3	49.1 ± 8.7	0.390	53.9 ± 10.2	53.3 ± 8.8	0.016
Education (less than middle school)	206 (9.9%)	286 (13.3%)	<0.001	525 (11.9%)	304 (16.4%)	<0.001
Reproductive Risk Factors						
Age at menarche (years)	14.5 ± 1.7	14.7 ± 1.8	<0.001	14.4 ± 1.7	14.7 ± 1.8	<0.001
Postmenopausal	804 (38.8%)	873 (40.7%)	0.195	2,257 (51.1%)	1,047 (56.6%)	<0.001
Age at menopause (years) ¹	48.4 ± 4.5	47.7 ± 4.8	0.002	49.0 ± 4.3	48.8 ± 4.1	0.162
Age at first live birth (years) ²	26.5 ± 3.9	26.0 ± 3.8	0.001	26.8 ± 3.9	25.5 ± 4.0	<0.001
Use of oral contraceptives ³	419 (20.2%)	433 (20.2%)	0.997	166 (17.2%)	416 (22.5%)	0.001
Additional Risk Factors						
First degree relative with breast cancer	92 (4.4%)	63 (2.9%)	0.010	246 (5.6%)	24 (2.6%)	<0.001
Use of hormone replacement therapy ⁴	65 (3.1%)	51 (2.4%)	0.136	161 (5.7%)	70 (3.8%)	0.003
History of breast fibroadenomas ⁵	201 (9.7%)	121 (5.6%)	<0.001	96 (10.0%)	48 (5.1%)	<0.001
Body mass index (kg/m ²)	23.8 ± 3.3	23.3 ± 3.4	<0.001	24.0 ± 3.5	23.6 ± 3.3	<0.001
Waist-to-hip ratio	0.82 ± 0.06	0.81 ± 0.06	<0.001	0.83 ± 0.05	0.82 ± 0.05	<0.001
Regular physical activity	521 (25.1%)	638 (29.7%)	<0.001	2,473 (56.0%)	622 (33.6%)	<0.001

Continuous variables: mean values ± standard deviation, p-value from t-tests; Categorical variables: numbers and percentages, p-values from χ^2 test

¹ Among postmenopausal women

² Among parous women

³ Information unavailable for 3,456 participants genotyped in Stage 2

⁴ Information unavailable for 1,601 participants genotyped in Stage 2

⁵ Information unavailable for 4,375 participants genotyped in Stage 2

Bold values considered to be significant p 0.05

Table 2
MMP-9 Polymorphisms and Breast Cancer Risk, The Shanghai Breast Cancer Genetics Study

SNP	Alleles *	Region	Method [†]	HWE [‡]	MAF (%) ^{‡‡}	Cases / Controls [§]	Breast Cancer Risk ^{§§}		
							AB OR (95% CI)	BB OR (95% CI)	p-value
Stage 1									
rs6065912	A/G	promoter	Affy 6.0	0.725	6.9	2,064 / 2,081	1.0 (0.9–1.2)	0.8 (0.3–2.0)	0.884
rs4810482	C/T	promoter	Targeted	0.270	28.1	1,056 / 1,064	1.1 (0.9–1.3)	1.0 (0.7–1.3)	0.802
rs3918241	T/A	promoter	Targeted	0.310	12.5	1,054 / 1,064	1.1 (0.9–1.3)	1.8 (0.9–3.6)	0.116
rs3918249	T/C	promoter	Affy 6.0	0.703	26.9	1,908 / 1,919	1.1 (1.0–1.2)	1.1 (0.9–1.5)	0.148
rs17576	G/A	exon 6 (279 Q/R)	Targeted	0.395	28.2	1,058 / 1,063	1.0 (0.9–1.2)	1.0 (0.7–1.3)	0.905
rs2250889	C/G	exon 10 (574 R/P)	Targeted	0.334	24.8	1,056 / 1,062	1.0 (0.9–1.2)	0.9 (0.7–1.3)	0.990
rs2274756	G/A	exon 12 (668 R/Q)	Targeted	0.254	12.3	1,054 / 1,064	1.1 (0.9–1.4)	2.0 (1.0–4.0)	0.056
Stage 2									
rs3918241	T/A	promoter	Targeted	0.303	11.6	4,354 / 1,835	1.1 (1.0–1.3)	1.1 (0.7–1.6)	0.222
Combined									
rs3918241	T/A	promoter	Targeted	0.733	11.9	5,408 / 2,899	1.1 (1.0–1.2)	1.2 (0.8–1.8)	0.051

^{*} Major/minor alleles as determined by allele frequency among genotyped controls

[†] Genotyping Method: Stage 1: Affymetrix Targeted Genotyping among 1,062 cases and 1,069 controls from SBCS I (Targeted), or Affymetrix 6.0 genotyping among 1,104 cases and 1,109 controls from SBCS I and 969 cases and 975 controls from SBCS II (Affy 6.0); Stage 2: Sequenom Targeted genotyping among 3,370 cases and 882 controls from SBCS/SECS, as well as 11 cases and 60 controls from SBCS I and 973 cases and 893 controls from SBCS II not previously genotyped (Targeted)

[‡] Hardy-Weinberg equilibrium p-value

^{‡‡} Minor allele frequency among genotyped controls

[§] Number of cases and controls successfully genotyped and included in breast cancer risk model

^{§§} Risk of breast cancer, adjusted for age, education, and study stage when appropriate; AA major allele homozygotes (reference group), AB heterozygotes, BB minor allele homozygotes; p-value for trend