Common MMP-7 Polymorphisms and Breast Cancer Susceptibility: A Multistage Study of Association and Functionality

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Abstract

Matrix metalloproteinase-7 (MMP-7) is a small secreted proteolytic enzyme with broad substrate specificity against ECM and non-ECM components. Known to be vital for tumor invasion and metastasis, accumulating evidence also implicates MMP-7 in cancer development. Using data from the Shanghai Breast Cancer Study, we conducted a two-stage study to evaluate the association of MMP-7 single nucleotide polymorphisms (SNPs) with breast cancer risk. Additionally, associated SNPs were characterized by laboratory assays. In stage 1, 11 SNPs were genotyped among 1,079 incident cases and 1,082 community controls using an Affymetrix Genotyping System. Promising SNPs were selected for stage 2 evaluation and genotyped by TaqMan allelic discrimination assays in an approximate 2- to 3-fold elevated level of protein expression (19). In transient transfection assays, the presence of both minor alleles conferred an approximately 2- to 3-fold elevated level of protein expression (19). In population studies, the MMP-7 –rs11568818 G allele has been shown to be associated with susceptibility for several cancers, including esophageal, gastric, lung, colorectal, and ovarian carcinomas (20–23). To our knowledge, MMP-7 polymorphisms have not been evaluated in relation to breast cancer risk. In the present study, we systematically searched for and evaluated common genetic polymorphisms in the MMP-7 gene in relation to breast cancer risk using data from a population-based case-control study involving 2,990 breast cancer cases and 2,893 controls. Furthermore, SNPs found to be associated with breast cancer were functionally characterized by in vitro experiments.

Materials and Methods

Study Design and Population

A two-stage (stage 1 and stage 2) case-control study design was used to first comprehensively evaluate MMP-7 polymorphisms in relation to breast cancer risk and then to validate promising associations in a second independent population study. Study subjects were participants of the Shanghai Breast Cancer Study (SBCS), a population-based case-control study among women in urban Shanghai; detailed information on the study design and data collection procedures has previously described (24). Briefly, stage 1 cases were women diagnosed with breast cancer between August 1996 and March 1998, ages 25 to 64, without a previous cancer diagnosis, and alive at the time of interview. Recruitment for stage 2 occurred between April 2002 and February 2005, and eligibility criteria were expanded to include women up to age 70 (25). Cases were identified via the population-based Shanghai Cancer Registry; diagnoses were confirmed by two senior pathologists. Controls were randomly selected from the general population using the Shanghai Resident Registry, a population registry of adult residents in urban Shanghai; women with previous cancer diagnoses were excluded. Structured questionnaires were used to obtain detailed information on demographic, reproductive, and behavioral factors. Of eligible participants, 1,459 (91.1%) cases and 1,556 (90.3%) controls, and 1,989 cases (83.7%) and 1,989 controls (70.4%)...
completed in-person interviews for stage 1 and stage 2, respectively. In stage 1, 1,193 cases (81.8%) and 1,310 controls (84.2%) donated blood samples. In stage 2, 1,932 (97.1%) cases and 1,857 (93.4%) controls donated blood samples. In stage 2 SNPsranged from 92.4% to 40.2% 83.4 16.2 0.5 0.235 82.7 16.9 0.5 0.184 23.1% 59.2 35.4 5.4 0.880 54.7 38.9 6.4 0.637 49.5% 26.3 48.4 5.7 0.874 53.4 39.7 7.0 0.709 0.0% 100.0 0.0 0.0 NA 99.9 0.1 0.0 0.988 Twelve SNPs were selected, including two SNPs and genotype distributions among 2,161 stage 1 SBCS participants.

### Table 1. Description of MMP-7 SNPs and genotype distributions among 2,161 stage 1 SBCS participants

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene region</th>
<th>Major/Minor allele*</th>
<th>Minor allele frequency†</th>
<th>Control genotypes (n = 1,082)</th>
<th>Case genotypes (n = 1,079)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs880197</td>
<td>promoter</td>
<td>A/T</td>
<td>40.2%</td>
<td>34.7</td>
<td>38.7</td>
</tr>
<tr>
<td>rs7098318</td>
<td>promoter</td>
<td>G/A</td>
<td>8.6%</td>
<td>83.4</td>
<td>82.9</td>
</tr>
<tr>
<td>rs11568818</td>
<td>promoter</td>
<td>A/G</td>
<td>8.6%</td>
<td>83.2</td>
<td>82.7</td>
</tr>
<tr>
<td>rs11568819</td>
<td>promoter</td>
<td>C/T</td>
<td>0.0%</td>
<td>100.0</td>
<td>NA</td>
</tr>
<tr>
<td>rs11225307</td>
<td>intron 3</td>
<td>A/G</td>
<td>24.9%</td>
<td>56.8</td>
<td>56.7</td>
</tr>
<tr>
<td>rs17352054</td>
<td>intron 5</td>
<td>A/C</td>
<td>11.7%</td>
<td>77.8</td>
<td>77.9</td>
</tr>
<tr>
<td>rs495041</td>
<td>3' FR</td>
<td>C/T</td>
<td>49.5%</td>
<td>26.3</td>
<td>25.6</td>
</tr>
<tr>
<td>rs10895304</td>
<td>3' FR</td>
<td>A/G</td>
<td>24.1%</td>
<td>57.5</td>
<td>53.4</td>
</tr>
<tr>
<td>rs7935378</td>
<td>3' FR</td>
<td>T/C</td>
<td>23.1%</td>
<td>59.2</td>
<td>54.7</td>
</tr>
<tr>
<td>rs12184413</td>
<td>3' FR</td>
<td>C/T</td>
<td>27.4%</td>
<td>52.4</td>
<td>54.3</td>
</tr>
<tr>
<td>rs11225297</td>
<td>3' FR</td>
<td>A/T</td>
<td>19.1%</td>
<td>65.6</td>
<td>66.8</td>
</tr>
</tbody>
</table>

Abbreviations: AA, major allele homozygotes; BB, minor allele homozygotes, AB, heterozygotes; FR, flanking region.

*Major and minor alleles determined by the distribution among SBCS stage 1 controls.
†MAF among SBCS stage 1 controls.  
‡P, Hardy-Weinberg equilibrium test.
§1/3 region flanking the MMP-7 gene.

### In vitro Functional Experiments

MMP-7 reporter constructs and luciferase assays. To evaluate the function of SNPs located in the 3' flanking region of the MMP-7 gene, a series of experiments were conducted. First, a 3.5 kb fragment that included rs12184413 and rs11225297 was generated by PCR; the forward primer 5'-CACACACATTTTTAGGTCCTCCCAG-3' and backward primer 5'-CATCCTAGGCTAGGCTAGTGTGGTTG-3' were used. Template DNA from individuals known to have either the major or minor alleles for rs12184413 and rs11225297 was used; resulting fragments were cloned into a pGL3-Promoter vector (Promega). All DNA constructs were verified by sequencing. Transfection was performed with the use of FuGene 6 Transfection Reagent (Roche Diagnostics) in triplicate for each construct. MDA231 and MCF7 cells (2 x 10^5 each) were seeded in 24-well plates and cotransfected with pGL3-Reporter vector, a Renilla expressing vector to serve as a reference for transfection efficiency, and the different allele combination containing luciferase reporter constructs. Thirty-six to 48 h later, cells were lysed with Passive Lysis Buffer and luminescence (relative light units) was measured using the Dual-Luciferase Assay System (Promega). Reporter activity was measured as the ratio of firefly to Renilla luciferase activity, and results from five independent experiments were averaged.

### Electrophoretic mobility shift assay.

Biotin-labeled, double-stranded oligonucleotide probes containing sequences surrounding rs12184413 and rs11225297 were synthesized. Probes for the major and minor alleles for the two SNPs were 5'-GATAGGGTATGGGATCTGCGGAGTAAGAC-3' and 5'-GATAGGGTTAGGGATCTGCGGAGTAAGAC-3', respectively. Probes were incubated with nuclear protein extracts from MDA-MB-231 and MCF-7 breast cancer cells, in the presence or absence of competitors, i.e., unlabeled double-stranded probe. Protein-DNA complexes were resolved by PAGE and then detected using a LightShift Chemiluminescent EMSA kit (Pierce Biotechnology).

### Statistical Analysis

Hardy-Weinberg equilibrium (HWE) was tested by comparing the observed and expected genotype frequencies (χ² test) for the cases and controls separately. Characteristics between cases and controls were compared with the χ² test or Student's t test for categorical or continuous variables, respectively. Odds ratios (OR) and corresponding 95% confidence intervals (95% CI) were determined by logistic regression analyses; additive,
dominant, and recessive models were applied. Covariates considered included age at diagnosis, age at menarche, age at first live birth among parous women, age at menopause among postmenopausal women, history of breast fibroadenomas, body mass index (BMI), and leisure physical activity in the decade preceding diagnosis. Tests for trend were conducted by coding for the number of variant alleles (0, 1, 2) and reporting the activity in the decade preceding diagnosis. Tests for trend were conducted by coding for the number of variant alleles (0, 1, 2) and reporting the value from trend test from additive models of effect. P values for trends are shown. All statistical tests were two tailed, and P values of ≤0.05 were interpreted as statistically significant.

**Results**

Table 2 presents demographic, reproductive, and other known breast cancer risk factors by case-control status for the SBCS participants included in the current study. As with studies conducted elsewhere (32, 33), early age at menarche, late age at menopause, and higher levels of regular physical activity were associated with lower breast cancer risk. Breast cancer risk was also positively associated with a history of breast fibroadenomas, obesity (higher body mass index), and higher rates of current smoking. The following factors were associated with lower breast cancer risk: a history of oral contraceptive use, regular physical activity, and use of estrogen replacement therapy after menopause.

**Table 2.** Description of demographic characteristics and known breast cancer risk factors in the SBCS

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Stage 1 (n = 2,161)</th>
<th>Stage 2 (n = 3,722)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases (n = 1,079)</td>
<td>Controls (n = 1,082)</td>
</tr>
<tr>
<td>Demographic factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>47.5 ± 7.9</td>
<td>47.0 ± 8.7</td>
</tr>
<tr>
<td>Education (less than middle school)</td>
<td>131 (12.1%)</td>
<td>161 (14.9%)</td>
</tr>
<tr>
<td>Reproductive risk factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at menarche (y)*</td>
<td>14.5 ± 1.6</td>
<td>14.7 ± 1.7</td>
</tr>
<tr>
<td>Age at menopause (y)*</td>
<td>48.1 ± 4.7</td>
<td>47.2 ± 5.0</td>
</tr>
<tr>
<td>Age at first live birth (y)*</td>
<td>26.9 ± 4.1</td>
<td>26.2 ± 3.8</td>
</tr>
<tr>
<td>Used oral contraceptives</td>
<td>230 (21.3%)</td>
<td>229 (21.2%)</td>
</tr>
<tr>
<td>Used estrogen replacement therapy</td>
<td>29 (2.7%)</td>
<td>28 (2.6%)</td>
</tr>
<tr>
<td>Additional Risk Factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First degree relative with breast cancer</td>
<td>37 (3.4%)</td>
<td>30 (2.8%)</td>
</tr>
<tr>
<td>Ever had breast fibroadenomas</td>
<td>106 (9.8%)</td>
<td>50 (4.6%)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.6 ± 3.4</td>
<td>23.3 ± 3.4</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.8 ± 0.06</td>
<td>0.80 ± 0.06</td>
</tr>
<tr>
<td>Regular physical activity</td>
<td>208 (19.3%)</td>
<td>274 (25.4%)</td>
</tr>
</tbody>
</table>

**NOTE:** Continuous variables: mean values ± SD. P value from Student’s t tests; Categorical variables: numbers and percentages. P values from χ² test. Bold values considered to be significant P < 0.05.

*Among postmenopausal women.

† Among parous women.

Table 3. Risk of breast cancer associated with *MMP-7* SNPs in the SBCS (stage 1)

<table>
<thead>
<tr>
<th>SNP</th>
<th>All women (1,079/1,082)*</th>
<th>Premenopausal (727/701)*</th>
<th>Postmenopausal (352/381)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>p</td>
<td>p</td>
</tr>
<tr>
<td>rs880197</td>
<td>A/T</td>
<td>0.9 (0.7–1.0)</td>
<td>0.8 (0.6–1.1)</td>
</tr>
<tr>
<td>rs17098318</td>
<td>G/A</td>
<td>1.0 (0.8–1.3)</td>
<td>0.8 (0.2–2.8)</td>
</tr>
<tr>
<td>rs11568818</td>
<td>A/G</td>
<td>1.0 (0.8–1.3)</td>
<td>1.0 (0.3–3.3)</td>
</tr>
<tr>
<td>rs11225307</td>
<td>A/G</td>
<td>1.0 (0.8–1.2)</td>
<td>1.0 (0.7–1.4)</td>
</tr>
<tr>
<td>rs117352054</td>
<td>A/C</td>
<td>1.0 (0.8–1.2)</td>
<td>1.4 (0.6–2.9)</td>
</tr>
<tr>
<td>rs495041</td>
<td>C/T</td>
<td>1.2 (1.0–1.4)</td>
<td>1.1 (0.9–1.4)</td>
</tr>
<tr>
<td>rs10985304</td>
<td>A/G</td>
<td>1.2 (1.0–1.4)</td>
<td>1.3 (0.9–1.8)</td>
</tr>
<tr>
<td>rs7935378</td>
<td>T/C</td>
<td>1.2 (1.0–1.4)</td>
<td>1.3 (0.9–1.8)</td>
</tr>
<tr>
<td>rs12184413</td>
<td>C/T</td>
<td>1.0 (0.8–1.2)</td>
<td>0.7 (0.5–1.0)</td>
</tr>
<tr>
<td>rs11225297</td>
<td>A/T</td>
<td>1.0 (0.8–1.2)</td>
<td>0.9 (0.6–1.4)</td>
</tr>
</tbody>
</table>

**NOTE:** NA, unstable estimates due to small genotype counts. Bold values considered to be significant P < 0.05.

*No of cases/controls.

† AA reference group; estimates for AB genotype, adjusted for age and education level.

‡ AA reference group; estimates for BB genotype, adjusted for age and education level.

P value from trend test from additive models of effect.
Table 4. Haplotype analysis of MMP-7 SNPs and breast cancer risk among the stage 1 SBCS participants

<table>
<thead>
<tr>
<th>Haplotype*</th>
<th>Frequency</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block 1: rs880197, rs17098318, rs11568818, rs11225307, rs17352054</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1: TGAAA</td>
<td>40.2</td>
<td>1.0</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>H2: AGAAA</td>
<td>26.2</td>
<td>1.2</td>
<td>1.0–1.4</td>
<td>0.033</td>
</tr>
<tr>
<td>H3: AGAGA</td>
<td>13.4</td>
<td>1.0</td>
<td>0.9–1.3</td>
<td>0.622</td>
</tr>
<tr>
<td>H4: AGAGC</td>
<td>11.5</td>
<td>1.1</td>
<td>0.9–1.3</td>
<td>0.427</td>
</tr>
<tr>
<td>H5: AAGAA</td>
<td>8.5</td>
<td>1.1</td>
<td>0.9–1.4</td>
<td>0.458</td>
</tr>
</tbody>
</table>

In rs495041, rs10895304, rs7935378

| H1: CAT | 46.6 | 1.0 | Reference | |
| H2: TAT | 28.0 | 1.0 | 0.9–1.2 | 0.987 |
| H3: TGC | 19.0 | 1.2 | 1.0–1.4 | 0.028 |

Block 2: rs12184413, rs11225297

| H1: C4 | 71.9 | 1.0 | Reference | |
| H2: TT | 18.6 | 0.9 | 0.8–1.1 | 0.381 |
| H3: T4 | 8.9 | 0.9 | 0.7–1.1 | 0.176 |

10 Polymorphic MMP-7 SNPs

| H1: TGAAATCA | 23.4 | 1.0 | Reference | |
| H2: AGAAFGCCA | 17.5 | 1.3 | 1.1–1.5 | 0.012 |
| H3: TGAAACATCA | 12.9 | 1.0 | 0.8–1.3 | 0.889 |
| H4: AGAGGATT | 9.8 | 1.1 | 0.9–1.4 | 0.395 |
| H5: AGAGACATT | 6.8 | 1.1 | 0.9–1.4 | 0.391 |
| H6: AAGASATCA | 5.7 | 1.1 | 0.8–1.5 | 0.518 |

NOTE: Bold values considered to be significant P < 0.05.
* Bold letters indicate less common alleles.
† Frequency of haplotype among controls.
‡ Age and education adjusted estimates from additive models.
§ P value from trend test.

menopause, late age at first live birth, use of estrogen replacement therapy, having a first-degree relative with breast cancer, prior history of fibroadenomas, high BMI or waist-to-hip ratio, and less regular physical activity were found to be associated with the risk of breast cancer in this study. Women participating in stage 1 and stage 2 were comparable in all listed characteristics, with the exceptions of age and leisure activity; these differences resulted from the expansion of the inclusion criteria between the two study stages.

Descriptive information on the 11 SNPs evaluated in stage 1 is shown in Table 1. One SNP (rs11568819) was not found to be polymorphic in our study population. None of the genotype distributions of the remaining 10 SNPs deviated from HWE.

Additive models assessing the association between MMP-7 polymorphisms and breast cancer for stage 1 are shown in Table 3. Estimates included adjustment for age and education; additional adjustment for age at menarche, age at menopause if postmenopausal, age at first live birth if parous, history of breast fibroadenomas, BMI, and leisure physical activity in the past 10 years did not appreciably alter the estimates of effect and were therefore not included. The rare allele of rs880197 was less common among breast cancer cases, and the gene-dose association was of borderline significance (P = 0.07). Homozygotes for the rs12184413 variant allele had a decreased risk of breast cancer, particularly among postmenopausal women (OR, 0.4; 95% CI, 0.2–0.9). On the other hand, having a rare allele for either rs10895304 or rs7935378 was associated with increased breast cancer risk among premenopausal women, and the trend tests for both SNPs was statistically significant (P < 0.02). Premenopausal homozygotes for either rs10895304 or rs7935378 were at a significantly increased risk of breast cancer (OR, 1.9; 95% CI, 1.2–3.0, for either SNP), whereas postmenopausal homozygotes had nonsignificant reductions in risk (rs10895304: OR, 0.7; 95% CI, 0.4–1.2; and rs7935378: OR, 0.6; 95% CI, 0.3–1.2). In stage 1, only 2.7% (n = 29) of patients were diagnosed with in situ carcinomas; excluding these patients did not appreciably alter the results (data not shown).

Based on the LD structure of the MMP-7 gene among the SBCS stage 1 controls (Supplementary Fig. S1), there are two haplotype blocks for this gene, one consisting of three promoter and two intronic SNPs, the other of two 3‘ FR SNPs. Haplotype analysis, based on additive models of effect, is shown in Table 4. The 5 SNPs in block 1 generated 5 common haplotypes, which accounted for 99.8% of all block 1 haplotypes. The second (H2: AGAA4) was associated with a significant increase in breast cancer risk (OR, 1.2; 95% CI, 1.1–1.5) compared with the reference haplotype (H1: TGAAA) that contained the variant allele of rs880197. This is in agreement with single SNP analysis, as the rare allele for rs880197 (T) tended to have a decreased risk of breast cancer compared with the common allele. Analysis of the 3 SNPs in between blocks 1 and 2 yielded 3 common haplotypes, accounting for 93.6% of all haplotypes in this region. The third (H3: TGC) had the rare alleles for rs495041, rs10895304, and rs7935378 and was associated with an increased breast cancer risk (OR, 1.2; 95% CI, 1.0–1.4), which is also consistent with the single marker analyses shown in Table 3. No significant associations were seen for block 2.
although both haplotypes with the variant allele of rs12184413 tended to have reduced risk compared with the reference haplotype. This is not surprising, as the single marker association for this SNP was not additive, but only evident among homozygotes. When all 10 MMP-7 SNPs were included, the significant findings above were better explained. The two haplotypes with increased risk (block 1, H2: AGAAAA, and in between SNPs, H3: TGC), were found to occur together (H2: AGAAACTGCA). Haplotypes that differed from the reference haplotype by three polymorphisms (rs880197, rs10895304, and rs7935378) had an ~30% greater risk of breast cancer (OR, 1.3; 95% CI, 1.1–1.5). Recessive models for haplotype effects were not permitted, as estimates of effect were unstable. Similarly, analyses stratified by menopausal status for less common haplotypes were not permitted. Interactions with menopausal status were evaluated for common haplotypes, and no significant interactions were observed (data not shown).

Of the 4 promising SNPs revealed from the single marker and haplotype analysis presented above, 2 (rs10895304 and rs7935378) were in high LD (D = 92%; r² = 0.81); rs10895304 was selected for stage 2 validation because it had a slightly greater MAF. Also included in stage 2 was rs880197 in the promoter and rs12184413 in the 3’ flanking region. After genotyping ~1,000 samples, preliminary analysis for stage 2 was conducted. Results from rs880197 for an association with breast cancer were in the opposite direction of those seen in stage 1 (data not shown), indicating that the findings for this SNP in stage 1 were unlikely to be replicated in stage 2. Therefore, this SNP was not further evaluated. Stage 2 genotyping was completed for rs10895304 and rs12184413. The results for rs10895304 were unsupportive of the stage 1 hypothesis; no association was seen (OR, 1.0; 95% CI, 0.8–1.1 for heterozygotes; OR, 1.0; 95% CI, 0.7–1.3 for homozygotes). Results for rs12184413 showed an association in agreement with stage 1, as shown in Table 5. A reduced risk of breast cancer was found to be associated with the TT genotype (OR, 0.7) in both stage 1 and stage 2. Only 2.1% (n = 39) of stage 2 patients were diagnosed with in situ carcinomas; their exclusion from the analyses did not materially alter the results (data not shown). In combined analysis of the two stages of the study, women homozygous for the rare allele had a significantly decreased risk of breast cancer (OR, 0.7; 95% CI, 0.6–0.9). The reduction in risk associated with this SNP was consistent with a recessive effect and was more pronounced in postmenopausal women (OR, 0.6; 95% CI, 0.4–0.8).

![Figure 1. Electrophoretic mobility shift assay for rs12184413 alleles.](image)

Table 5. Association of MMP-7 rs12184413 with breast cancer risk, the SBCS

<table>
<thead>
<tr>
<th></th>
<th>Additive models</th>
<th></th>
<th>Recessive models</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)*</td>
<td>OR (95% CI)†</td>
<td>P†</td>
<td>OR (95% CI)‡</td>
</tr>
<tr>
<td>Stage 1 (1,078/1,082)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All women</td>
<td>1.0 (0.8–1.2)</td>
<td>0.7 (0.5–1.0)</td>
<td>0.175</td>
<td>0.7 (0.5–1.0)</td>
</tr>
<tr>
<td>Premenopausal</td>
<td>0.9 (0.8–1.2)</td>
<td>0.9 (0.6–1.4)</td>
<td>0.478</td>
<td>0.9 (0.6–1.4)</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>1.0 (0.8–1.4)</td>
<td>0.4 (0.2–0.9)</td>
<td>0.170</td>
<td>0.4 (0.2–0.8)</td>
</tr>
<tr>
<td>Stage 2 (1,885/1,795)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All Women</td>
<td>1.0 (0.9–1.2)</td>
<td>0.7 (0.6–0.9)</td>
<td>0.200</td>
<td>0.7 (0.6–0.9)</td>
</tr>
<tr>
<td>Premenopausal</td>
<td>1.0 (0.8–1.2)</td>
<td>0.7 (0.5–1.1)</td>
<td>0.174</td>
<td>0.8 (0.5–1.1)</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>1.1 (0.9–1.4)</td>
<td>0.7 (0.5–1.1)</td>
<td>0.710</td>
<td>0.7 (0.5–1.0)</td>
</tr>
<tr>
<td>Combined (2,963/2,877)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All Women</td>
<td>1.0 (0.9–1.1)</td>
<td>0.7 (0.6–0.9)</td>
<td>0.071</td>
<td>0.7 (0.6–0.9)</td>
</tr>
<tr>
<td>Premenopausal</td>
<td>1.0 (0.8–1.1)</td>
<td>0.8 (0.6–1.1)</td>
<td>0.145</td>
<td>0.8 (0.6–1.1)</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>1.1 (0.9–1.3)</td>
<td>0.6 (0.5–0.9)</td>
<td>0.294</td>
<td>0.6 (0.4–0.8)</td>
</tr>
</tbody>
</table>

NOTE: Bold values considered to be significant P < 0.05.
* CC reference group; estimates for CT genotype, adjusted for age and education level.
† CC reference group; estimates for TT genotype, adjusted for age and education level.
‡ P value from trend test.
§ CC/CT reference group, estimates for TT genotype, adjusted for age and education level; P value for recessive association.
|| No of cases/number of controls.

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The rs12184413 SNP is located ∼1.7 kb downstream of the MMP-7 gene and is within a predicted potential regulatory region spanning 1.3 to 2.1 kb downstream of the gene (34). This purported CCCTC-binding factor (CTCF)-enriched binding site has been implicated in several forms of transcriptional regulation, including activation, repression, and genomic insulation (35, 36). To evaluate if this downstream region influences MMP-7 gene expression, in vitro luciferase assays were conducted in nonmetastatic MCF-7 and metastatic MDA-MB-231 breast cancer cells with reporter constructs containing the major and minor allele combinations of two SNPs, rs12184413 and rs11225297. Although a significant difference between constructs with the different allele combinations was not detected (data not shown), there was an average 10% decrease in luciferase activity from all constructs that included the 3.5 kb region from downstream of the MMP-7 gene (data not shown). To further investigate whether rs12184413 or rs11225297 interacts with nuclear proteins in breast cancer cells, and if so, whether a single nucleotide change in these sites can alter the protein-DNA interactions, we performed electrophoretic mobility shift assays. Results clearly show a difference in binding between the rs12184413 major and minor alleles (Fig. 1). The variant rs12184413 (T) resulted in weaker DNA-protein complex intensity compared with that of the major allele (C) in both MCF-7 and MDA-MB-231 cells, whereas no differential binding resulted from the different alleles of rs11225297 (data not shown).

Discussion

We evaluated common genetic variation in the MMP-7 gene in association with breast cancer risk in a large, population-based, two-stage case-control study of Chinese women, including 2,990 breast cancer cases and 2,893 controls. Women with rs12184413 TT were 30% less likely to be breast cancer cases than women with the CT and CC genotypes. Furthermore, this SNP was found to affect DNA-protein interactions such that the T allele had decreased protein binding. These findings are novel and suggest that common MMP-7 polymorphisms may contribute to the susceptibility of breast cancer.

Previous studies on MMP-7 genetic variation and cancer susceptibility have focused on two promoter polymorphisms that have been shown to affect expression (19). These studies have generally found positive associations with the risk of cancer (20, 21, 23, 37). A small Italian study reported that the rare alleles for both rs11568818 (−181 A/G) and rs11568819 (−153 C/T) occurred more frequently among colorectal cancer cases than controls (20). A small study among Han Chinese found that rs11568818 was associated with esophageal squamous cell carcinoma, gastric cardiac carcinoma, and non–small cell lung carcinoma (21). A hospital-based study reported a positive association with astrocytoma among Han Chinese (37), and a similar positive association with epithelial ovarian cancer was found among almost 300 women from Northern China (23). On the contrary, one study found that the rs11568818 G allele was associated with a reduced risk of gastric cancer among 248 Caucasians (22). In our study, we did not find a significant association for this SNP, although an increase in risk was suggested among premenopausal women. The MAF of rs11568818 in our study was low (8.6%), which may have limited our power to detect an association. In addition, rs11568819 was not found to be polymorphic in our study population. Our genotype frequencies for these two SNPs agrees with other reports (20–23) and indicates they have lower MAFs among Asian populations compared with Caucasians.

Our finding of a significant association between rs12184413 and breast cancer risk is supported by several lines of evidence. First, it is unlikely that this association occurred by chance, as we analyzed two separate and large study populations. Additionally, in silico analyses indicated that the MMP-7 3′ flanking region is conserved across eutherian mammals and, therefore, may have some important functional role. The region surrounding rs12184413 was found to be enriched with CTCF binding sites, a factor implicated in several forms of regulation, including gene activation, repression, silencing, and genomic insulation (35, 36). Furthermore, in vitro analyses showed that the major and minor alleles of rs12184413 have different capacities to bind nuclear proteins, whereas no difference was observed for rs11225297. Thus, it is possible that the potential CTCF binding region is a transcriptional repressor, and that binding of a nuclear factor relinquishes this repression, thereby allowing expression. Alternately, this region may be a genomic insulator, separating the chromosome 11 MMP gene cluster from other genes further away. In this case, CTCF binding would protect against the spreading of methylation (38), such that when unbound, MMP-7 expression would be reduced. Finally, we cannot exclude the possibility that rs12184413 may be in high LD with another region that is actually responsible for the alteration in cancer risk. In our data, this SNP was not in high LD with either of the known functional SNPs, and the two polymorphisms that were most linked were also both in the 3 flanking region: rs11225297 (D = 95%), and rs10895304 (D = 92%). Although the true sequence of interest may still be elsewhere in the MMP-7 gene, this seems unlikely as no tagging SNPs were indicative of such a relationship.

MMP-7 is a small secreted metalloproteinase that differs from most other MMPs in that it is expressed in epithelial rather than only stromal cells (1). MMP-7 is normally expressed in the breast and has been shown to contribute to the development and proper branching of the mammary system in mice (9, 39). In animals lacking MMP-7, reduced mammary lesions after chemical induction were found (7), while overexpression resulted in higher occurrences of mammary hyperplasia and accelerated tumor development (40). There are several mechanisms by which MMP-7 may contribute to cancer initiation and promotion. The MMPs provide regulatory signals for cellular adhesion, differentiation, division, and death; alterations in these pathways can cause loss of contact inhibition, aberrant cell growth, and evasion of apoptosis (4, 8). MMP-7 contributes to these events via the activation, degradation, and shedding of target molecules (4, 6). In addition to ECM substrates such as elastin, proteoglycans, fibronectin, type IV collagen, and E-cadherin (1), MMP-7 can degrade non-ECM components including Fas ligand, protumor necrosis factor-α, insulin-like growth factor binding proteins, and heparin-binding epidermal growth factor (1, 4, 6, 8). Resulting effects include potent mitogenic stimulation for cellular transformation and growth, as well as the dissociation of normal cellular organization, which may contribute to the epithelial-to-mesenchymal transition that occurs frequently during cancer progression (1, 6, 8). In addition, downstream effects of MMP-7 may also include either antiapoptotic signaling for increased cell survival, or stimulation for cell death, which is thought to select against susceptible
cells in the early tumor microenvironment and leave more robust cells to continue to grow and acquire malignant characteristics (4, 6, 8). In summary, MMP-7 has a myriad of functions that may contribute to the development and progression of cancer. Naturally occurring genetic variation has been previously shown to modify the expression of the MMP-7 gene and may thereby influence individual cancer risk. In a large two-stage case-control study of Chinese women, we found a significant association between a polymorphism 3’ downstream of the MMP-7 gene and a reduced risk of breast cancer. This association was detected in two independent and large population-based sets of study participants, and further, a functional difference for the alleles of this SNP was confirmed by in vitro experiments. Given the oncogenic effects of MMP-7 proteolysis, the decreased risk of breast cancer found, and the proposed 3’ regulatory region, these results not only show how small genetic differences may influence individual cancer susceptibility, but are also supportive of a role for MMP-7 in breast cancer etiology.

Disclosure of Potentials of Conflict
No potential conflicts of interest were disclosed.

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Common *MMP-7* Polymorphisms and Breast Cancer Susceptibility: A Multistage Study of Association and Functionality
