Null Results in Brief

No Association between Matrix Metalloproteinase-1 or Matrix Metalloproteinase-3 Polymorphisms and Breast Cancer Susceptibility: A Report from the Shanghai Breast Cancer Study

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Introduction

Matrix metalloproteinase (MMP)-1 (interstitial collagenase) and MMP-3 (stromelysin) are structurally related multifunctional enzymes that are involved in physiologic and pathologic tissue remodeling (1, 2). Known to contribute to breast cancer invasion and metastasis (3-5), roles in breast tumor initiation and progression have also been suggested (6-10). Expression of these MMPs is often coordinately regulated; the two genes are adjacent on chromosome 11q22.3 and have several similar promoter elements (11, 12). Functional polymorphisms resulting from the insertion or deletion of a single nucleotide have been identified in both gene promoters (13-15); MMP-1-1607 1G/2G (rs1799750) and MMP-3-1612 (also known as-1171) 5A/6A (rs35068180 and rs3025058) are in linkage disequilibrium (16, 17). Previous studies have evaluated these single nucleotide polymorphisms (SNP) in relation to breast cancer risk with both positive (18, 19) and null findings (20-22); however, other genetic variation in these genes may also contribute to expression differences (11, 12, 23). This study was therefore undertaken to comprehensively assess individual genetic variation across MMP-1 and MMP-3, and evaluate associations with breast cancer risk among participants of the Shanghai Breast Cancer Study.

Materials and Methods

Study subjects were participants of the Shanghai Breast Cancer Study, a large, two-phase, population-based, case-control study of women in urban Shanghai (24-26). Briefly, 1,459 (91.1%) cases and 1,556 (90.3%) controls from phase 1, and 1,989 cases (83.7%) and 1,989 controls (70.4%) from phase 2 completed in-person interviews. Blood or buccal cell samples were donated by 1,193 cases (81.8%) and 1,310 controls (97.1%) and 1,857 (93.4%) controls from phase 2. Approval was granted from relevant review boards in both China and the United States; all included subjects gave informed consent.

Haplotype tagging SNPs were selected from Han Chinese data from the HapMap Project (27) using the Tagger program (28) to capture SNPs with a minimum minor allele frequency (MAF) of 0.05 in either MMP-1 or MMP-3 (±5 kb) with an r² of 0.90 or greater. Seventeen MMP-1 and 7 MMP-3 SNPs were selected; 14 and 6 SNPs, respectively, were successfully designed and genotyped in 2006 for 1,062 cases and 1,069 controls from phase 1, using a Targeted Genotyping System (Affymetrix; ref. 26).

Two insertion/deletion polymorphisms reported to be functional (13-15) were chosen for genotyping using the Sequenom MassARRAY System (Sequenom, Inc.) for 1,495 cases and 1,437 controls from phase 2. Blinded duplicate samples and negative controls were included; concordance rates between duplicates were ≥99.4%.

To increase the density of genetic markers in this study, data from our recently completed Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix) was included for an additional 11 MMP-1 (±10 kb) and 9 MMP-3 (±10 kb) SNPs for 2,994 participants, including 1,082 cases and 1,085 controls from phase 1, and 416 cases and 411 controls from phase 2.

Hardy-Weinberg equilibrium was tested by comparing the observed and expected genotype frequencies of the controls (χ² test). Odds ratios (OR) and corresponding 95% confidence intervals were determined by logistic regression analyses using additive models that included adjustment for age, education, and study phase if appropriate. Linkage disequilibrium was assessed by Haploview (29). All statistical tests were two-tailed, and P values were considered to be statistically significant when ≤0.05.
Results

A total of 6,023 women were included in the current study: 2,279 phase 1 participants and 3,744 phase 2 participants. Women in both study phases were generally comparable (data not shown). As expected, breast cancer cases were found to differ from controls with regard 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 history of breast cancer among a second-degree relative and/or a history of breast cancer among a first-degree relative who died during menopause, and were less likely to participate in regular physical activity than controls (data not shown).

Table 1. MMP-1 and MMP-3 SNPs and breast cancer risk, the Shanghai Breast Cancer Study

<table>
<thead>
<tr>
<th>Gene</th>
<th>Alleles</th>
<th>Region</th>
<th>Genotyping</th>
<th>MAF</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-3</td>
<td>rs615098</td>
<td>C/A</td>
<td>Promoter</td>
<td>Affy 6.0</td>
<td>14.5%</td>
<td>0.9 (0.7-1.1)</td>
</tr>
<tr>
<td></td>
<td>rs613804</td>
<td>C/T</td>
<td>Promoter</td>
<td>Affy 6.0</td>
<td>11.4%</td>
<td>0.9 (0.7-1.1)</td>
</tr>
<tr>
<td></td>
<td>rs17361668</td>
<td>T/A</td>
<td>Promoter</td>
<td>Affy 6.0</td>
<td>2.8%</td>
<td>1.0 (0.7-1.4)</td>
</tr>
<tr>
<td></td>
<td>rs619590</td>
<td>T/C</td>
<td>Promoter</td>
<td>Affy 6.0</td>
<td>10.5%</td>
<td>0.9 (0.7-1.1)</td>
</tr>
<tr>
<td></td>
<td>rs645419</td>
<td>G/A</td>
<td>Promoter</td>
<td>Targeted</td>
<td>32.3%</td>
<td>1.0 (0.9-1.2)</td>
</tr>
<tr>
<td></td>
<td>rs35068180</td>
<td>6A/5A</td>
<td>Promoter</td>
<td>Sequenom</td>
<td>15.4%</td>
<td>1.1 (1.0-1.4)</td>
</tr>
<tr>
<td></td>
<td>rs632478</td>
<td>C/A</td>
<td>Promoter</td>
<td>Targeted</td>
<td>32.4%</td>
<td>1.0 (0.8-1.2)</td>
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<tr>
<td></td>
<td>rs522616</td>
<td>A/G</td>
<td>Promoter</td>
<td>Targeted</td>
<td>38.7%</td>
<td>1.0 (0.8-1.2)</td>
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<tr>
<td></td>
<td>rs676260</td>
<td>G/A</td>
<td>Exon 2</td>
<td>Targeted</td>
<td>32.5%</td>
<td>1.0 (0.8-1.2)</td>
</tr>
<tr>
<td></td>
<td>rs650108</td>
<td>A/G</td>
<td>Intron 8</td>
<td>Targeted</td>
<td>39.5%</td>
<td>1.0 (0.9-1.3)</td>
</tr>
<tr>
<td></td>
<td>rs655403</td>
<td>G/T</td>
<td>Intron 6</td>
<td>Targeted</td>
<td>6.9%</td>
<td>0.9 (0.7-1.2)</td>
</tr>
<tr>
<td></td>
<td>rs639752</td>
<td>T/G</td>
<td>Intron 9</td>
<td>Affy 6.0</td>
<td>31.5%</td>
<td>1.1 (0.9-1.3)</td>
</tr>
<tr>
<td>MMP-1</td>
<td>rs2150013</td>
<td>C/T</td>
<td>Intron 4</td>
<td>Targeted</td>
<td>7.1%</td>
<td>1.1 (0.9-1.5)</td>
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<tr>
<td></td>
<td>rs502858</td>
<td>T/A</td>
<td>3' FR</td>
<td>Affy 6.0</td>
<td>49.6%</td>
<td>1.0 (0.9-1.3)</td>
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<tr>
<td></td>
<td>rs7926920</td>
<td>G/A</td>
<td>3' FR</td>
<td>Affy 6.0</td>
<td>32.1%</td>
<td>1.0 (0.9-1.2)</td>
</tr>
<tr>
<td></td>
<td>rs484915</td>
<td>A/T</td>
<td>promoter</td>
<td>Targeted</td>
<td>33.6%</td>
<td>0.9 (0.8-1.1)</td>
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<tr>
<td></td>
<td>rs1155764</td>
<td>T/G</td>
<td>Promoter</td>
<td>Targeted</td>
<td>20.1%</td>
<td>1.0 (0.9-1.3)</td>
</tr>
<tr>
<td></td>
<td>rs509332</td>
<td>A/G</td>
<td>Promoter</td>
<td>Targeted</td>
<td>12.8%</td>
<td>0.9 (0.7-1.1)</td>
</tr>
<tr>
<td></td>
<td>rs470206</td>
<td>G/A</td>
<td>Promoter</td>
<td>Targeted</td>
<td>12.7%</td>
<td>0.9 (0.7-1.1)</td>
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<tr>
<td></td>
<td>rs1799750</td>
<td>2G/1G</td>
<td>Promoter</td>
<td>Sequenom</td>
<td>34.9%</td>
<td>1.0 (0.8-1.2)</td>
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<tr>
<td></td>
<td>rs2075847</td>
<td>T/C</td>
<td>Promoter</td>
<td>Targeted</td>
<td>24.2%</td>
<td>1.0 (0.9-1.2)</td>
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<tr>
<td></td>
<td>rs1939008</td>
<td>A/G</td>
<td>2G/1G</td>
<td>Sequenom</td>
<td>34.9%</td>
<td>1.1 (0.9-1.3)</td>
</tr>
<tr>
<td></td>
<td>rs470504</td>
<td>C/T</td>
<td>3' FR</td>
<td>Affy 6.0</td>
<td>32.1%</td>
<td>1.0 (0.9-1.2)</td>
</tr>
<tr>
<td></td>
<td>rs7127735</td>
<td>A/G</td>
<td>3' FR</td>
<td>Affy 6.0</td>
<td>32.1%</td>
<td>1.0 (0.9-1.2)</td>
</tr>
</tbody>
</table>

Abbreviation: UTR, untranslated region.

1Genotyping: Affymetrix Targeted Genotyping among 1,062 cases and 1,069 controls from phase 1 (Targeted); Sequenom Targeted Genotyping among 1,495 cases and 1,437 controls from phase 2 (Sequenom); or Affymetrix 6.0 genotyping among 1,082 cases and 1,085 controls from phase 1 and 416 cases and 411 controls from phase 2 (Affy 6.0).

*Odds Ratio (OR) and corresponding 95% Confidence Interval (CI) for the risk of breast cancer, adjusted for age, education, and study phase (if appropriate); AA major allele homozygotes (reference group); AB heterozygotes; BB minor allele homozygotes; P value for trend from additive model. 

**FR, downstream flanking region, 3' of the gene.**
17 SNPs. None of these 39 SNPs were found to be significantly associated with breast cancer risk in additive models that included adjustment for age, education, and study phase when appropriate. Furthermore, no significant associations were identified under dominant or recessive models (data not shown). The linkage disequilibrium structure of these 39 polymorphic loci is shown in Fig. 1.

Discussion

Known to be involved in cancer invasion and metastasis, MMP-1 and MMP-3 have also been implicated in breast cancer development and progression. MMP-3 expression was found to promote malignant transformation in vitro and the development of spontaneous malignant lesions in mammary glands of mice (6-8). MMP-1 expression was necessary for breast tumor growth in nude mice (9), and was determined to be positively regulated by Her-2/neu induced Ets-1 in breast cancer cells (10). In humans, both genes were found to be expressed in breast cancer tissues (30). Promoter polymorphisms that influence gene expression have been identified for both MMP-1 and MMP-3 (13, 15, 25). Previous studies on MMP-1-1607 1G/2G (rs1799750) and MMP-3-1612 (aka -1171) 5A/6A (rs35068180 and rs3025058) and breast cancer risk have had mixed results (18-22). In the current study, neither the two previously reported functional SNPs, nor other genetic variation in or around MMP-1 or MMP-3, were found to be associated with breast cancer risk. Given the size of our study population, this analysis had >77% power to detect an OR of 1.3 for a SNP with a MAF of 10%, >85% power to detect an OR of 1.25 for a SNP with a MAF of 20%, and >79% power to detect an OR of 1.2 for a SNP with a MAF of 30%. In summary, a total of 42 MMP-1 and MMP-3 polymorphisms were evaluated among a total of 3,016 cases and 3,007 controls in the Shanghai Breast Cancer Study; no associations with breast cancer risk were observed.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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