Null Results in Brief

No Association between Matrix Metalloproteinase (MMP)-1, MMP-3, and MMP-7 SNPs and Endometrial Cancer Risk

Alicia Beeghly-Fadiel,1 Yong-Bing Xiang,2 Sandra L. Deming,1 Ji-Rong Long,1 Wang-Hong Xu,3 Qiuyin Cai,1 Wei Zheng,1 and Xiao Ou Shu1

1Vanderbilt University Medical Center, Division of Epidemiology, Nashville, Tennessee; and 2Shanghai Cancer Institute, Department of Epidemiology; and 3Fudan University School of Public Health, Department of Epidemiology, Shanghai, China

Introduction

The matrix metalloproteinases (MMP) function as regulators of the dynamic tissue remodeling that occurs in the endometrial lining of the uterus during the normal human menstrual cycle; dysregulation of the MMPs is thought to contribute to the development of both endometriosis and endometrial cancer (1). MMP-1 expression was found to be significantly higher in endometriotic lesions than in surrounding endometrium (2, 3), whereas MMP-3 levels have been reported to be both lower (4) and higher (5) in women with endometriosis compared with women without. MMP-7 expression was found to be significantly higher in endometrial hyperplasia and adenocarcinomas than normal endometrium (6) and, further, was associated with higher grade endometrial tumors (7), and both myometrial (6) and lymph node invasion (8). These three MMP genes are located on the negative strand of chromosome 11, and functional polymorphisms that influence their respective transcription levels have been identified for each (9-12). However, previous studies on MMP SNPs and endometrial cancer are sparse; two studies were found to have evaluated a single MMP-1 single nucleotide polymorphism (SNP) and results were inconsistent. Therefore, this comprehensive study of individual genetic variation across MMP-1, MMP-3, and MMP-7 was undertaken to evaluate associations with endometrial cancer susceptibility.

Materials and Methods

The Shanghai Endometrial Cancer Study (SECS) is a large, population-based case-control study that has been previously described (13, 14). Briefly, cases were women diagnosed with endometrial cancer between January 1997 and December 2003, ages 30 to 69 y, identified from the Shanghai Cancer Registry. Controls were randomly selected from the Shanghai Resident Registry and frequency matched to cases in 5-year intervals. Of 1,458 identified eligible cases, in-person interviews were completed for 1,204 (82.6%). Reasons for nonparticipation included refusal (n = 137, 9.4%), death before interview (n = 66, 4.5%), inability to be located (n = 37, 2.5%), and health or communication problems (n = 14, 1.0%). Of eligible controls identified (1,629), in-person interviews were completed for 1,212 (74.4%). Reasons for nonparticipation included refusal (n = 340, 20.9%), absence during the study period (n = 61, 3.7%), and health or communication problems (n = 16, 1.1%). Institutional review board approval was granted by relevant institutions in both China and the United States. Informed consent was obtained from each included participant. DNA samples were provided and available for 87.3% of cases (n = 1,052) and 87.3% (n = 1,058) of controls.

Haplotype-tagging SNPs were selected from Han Chinese data from the HapMap Project (15) using the Tagger program (16) to capture SNPs with a minimum minor allele frequency (MAF) of 0.05 in either MMP-1, MMP-3, or MMP-7 (± 5 kb) with an r2 of 0.90 or greater. Known or potentially functional SNPs were forced into the haplotype-tagging SNP selection process. For MMP-1, 17 SNPs were selected, with 14 successfully genotyped. For MMP-3, seven SNPs were selected, with six successfully genotyped. For MMP-7, 12 SNPs were selected, with 11 successfully genotyped. Genotyping was conducted using the Affymetrix Targeted Genotyping System (Affymetrix; ref. 17) for 1,037 cases (98.6%) and 1,018 controls (96.2%).

Hardy-Weinberg equilibrium was applied to test the observed and expected genotype frequencies for cases and controls (χ2 test). Associations between SNPs and covariates were evaluated with the χ2 test or t test when appropriate. Covariates considered included age at diagnosis, education, age at menarche, age at menopause among postmenopausal women, menopausal status, number of pregnancies, oral contraceptive use, body mass index, waist-to-hip ratio (WHR), physical activity in the preceding decade, and first-degree family history of breast, colorectal, or endometrial cancer. Odds ratios and
corresponding 95% confidence intervals were determined by logistic regression using additive models that included adjustment for age and education. Dominant and recessive models were additionally used when appropriate. Linkage disequilibrium was assessed by Haploview (18). All statistical tests were two-tailed, and \( P \) values were considered to be statistically significant when \( \leq 0.05 \).

## Results

Consistent with previous SECS analyses (13, 14) and other epidemiologic studies, cases, and controls included in the current study differed with regard to age at menarche, age at menopause, menopausal status, number of pregnancies, use of oral contraceptives, body mass index and WHR, physical activity, and first-degree family history of cancer (data not shown). SNPs included in this study are listed in Table 1; their order corresponds to the open reading frames of the genes on the negative strand of chromosome 11. Of the 31 polymorphisms genotyped, one was found not to be polymorphic in this study population (MMP-7 rs11568819) and thus was not included in our analyses. No SNPs were found to deviate from Hardy-Weinberg equilibrium. Associations with endometrial cancer risk were calculated in additive effect models that included adjustment for age and education; further adjustment for body mass index, number of pregnancies, menopausal status, or family history of cancer did not appreciably alter the effect estimates. No significant associations were observed. Two MMP-7 SNPs, rs17098318 and rs11568818, both tended to confer an increased, but nonsignificant, risk of endometrial cancer for homozygotes, in both additive and recessive models. Furthermore, no SNPs were found to have effects that significantly differed by menopausal status. The linkage disequilibrium structure of these 30 SNPs is shown in Fig. 1, and includes six haplotype blocks. Similar to single SNP analysis, no significant effects were observed in haplotype analysis of these MMP polymorphisms (data not shown).

## Discussion

Promoter polymorphisms in the MMP-1, MMP-3, and MMP-7 genes have been associated with altered susceptibility to cancer in human populations (17, 19-30), although studies on endometrial cancer risk and MMP SNPs have been lacking. Two small studies evaluating MMP-1 -1607 1G/2G (rs1799750) and MMP-3 -1171

<table>
<thead>
<tr>
<th>Gene, SNP Region</th>
<th>Alleles*</th>
<th>MAF†</th>
<th>HWE</th>
<th>Endometrial cancer risk, OR (95% CI)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-3 rs645419</td>
<td>Promoter G/A</td>
<td>32.6%</td>
<td>0.573</td>
<td>1.1 (0.9-1.3)</td>
</tr>
<tr>
<td></td>
<td>Promoter C/A</td>
<td>33.0%</td>
<td>0.766</td>
<td>1.1 (0.9-1.3)</td>
</tr>
<tr>
<td>rs632478</td>
<td>Promoter A/G</td>
<td>36.2%</td>
<td>0.700</td>
<td>1.0 (0.8-1.2)</td>
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<tr>
<td>rs522616</td>
<td>Promoter A/G</td>
<td>32.9%</td>
<td>0.754</td>
<td>1.1 (0.9-1.3)</td>
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<tr>
<td>rs679620</td>
<td>Intron 2 G/A</td>
<td>40.2%</td>
<td>0.903</td>
<td>1.1 (0.9-1.3)</td>
</tr>
<tr>
<td>rs650108</td>
<td>Intron 3 C/T</td>
<td>7.2%</td>
<td>0.552</td>
<td>1.0 (0.8-1.3)</td>
</tr>
<tr>
<td>MMP-1 rs484915</td>
<td>Promoter A/T</td>
<td>34.2%</td>
<td>0.311</td>
<td>1.0 (0.8-1.2)</td>
</tr>
<tr>
<td>rs5909332</td>
<td>Promoter A/T</td>
<td>22.0%</td>
<td>0.765</td>
<td>0.9 (0.7-1.0)</td>
</tr>
<tr>
<td>rs470206</td>
<td>Promoter A/G</td>
<td>13.4%</td>
<td>0.124</td>
<td>1.1 (0.9-1.4)</td>
</tr>
<tr>
<td>rs2075847</td>
<td>Promoter T/C</td>
<td>24.0%</td>
<td>0.114</td>
<td>1.1 (0.9-1.4)</td>
</tr>
<tr>
<td>rs498186</td>
<td>Promoter A/G</td>
<td>44.0%</td>
<td>0.253</td>
<td>1.2 (0.8-1.6)</td>
</tr>
<tr>
<td>rs475007</td>
<td>Promoter T/A</td>
<td>34.0%</td>
<td>0.966</td>
<td>1.0 (0.8-1.2)</td>
</tr>
<tr>
<td>rs996999</td>
<td>Intron 4 C/T</td>
<td>49.1%</td>
<td>0.702</td>
<td>0.9 (0.8-1.1)</td>
</tr>
<tr>
<td>rs470558</td>
<td>Exon 5 G/A</td>
<td>12.4%</td>
<td>0.642</td>
<td>0.9 (0.7-1.1)</td>
</tr>
<tr>
<td>rs7125062</td>
<td>Intron 6 C/T</td>
<td>30.6%</td>
<td>0.478</td>
<td>1.0 (0.8-1.2)</td>
</tr>
<tr>
<td>rs1938901</td>
<td>Intron 8 T/C</td>
<td>42.4%</td>
<td>0.691</td>
<td>1.1 (0.9-1.3)</td>
</tr>
<tr>
<td>rs2071231</td>
<td>Intron 9 T/G</td>
<td>20.4%</td>
<td>0.607</td>
<td>1.1 (0.9-1.3)</td>
</tr>
<tr>
<td>rs7945189</td>
<td>3' FR² G/A</td>
<td>13.9%</td>
<td>0.250</td>
<td>0.9 (0.7-1.1)</td>
</tr>
<tr>
<td>rs1477054</td>
<td>3' FR²</td>
<td>0.8%</td>
<td>0.663</td>
<td>0.9 (0.7-1.1)</td>
</tr>
<tr>
<td>MMP-7 rs880197</td>
<td>Promoter A/T</td>
<td>38.7%</td>
<td>0.755</td>
<td>0.9 (0.7-1.1)</td>
</tr>
<tr>
<td>rs17098318</td>
<td>Promoter G/A</td>
<td>7.9%</td>
<td>0.309</td>
<td>1.0 (0.8-1.2)</td>
</tr>
<tr>
<td>rs11568818</td>
<td>Promoter A/G</td>
<td>8.0%</td>
<td>0.281</td>
<td>1.0 (0.7-1.2)</td>
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<tr>
<td>rs1225307</td>
<td>Intron 3 A/G</td>
<td>25.0%</td>
<td>0.461</td>
<td>1.1 (0.9-1.3)</td>
</tr>
<tr>
<td>rs17352054</td>
<td>Intron 5 A/C</td>
<td>12.0%</td>
<td>0.682</td>
<td>1.2 (1.0-1.5)</td>
</tr>
<tr>
<td>rs495041</td>
<td>3' FR² C/T</td>
<td>49.5%</td>
<td>0.508</td>
<td>0.9 (0.8-1.2)</td>
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<tr>
<td>rs10893004</td>
<td>3' FR² A/G</td>
<td>24.5%</td>
<td>0.853</td>
<td>1.0 (0.8-1.2)</td>
</tr>
<tr>
<td>rs7925378</td>
<td>3' FR² T/C</td>
<td>25.0%</td>
<td>0.941</td>
<td>0.9 (0.8-1.1)</td>
</tr>
<tr>
<td>rs12184413</td>
<td>3' FR² C/T</td>
<td>29.5%</td>
<td>0.577</td>
<td>0.9 (0.8-1.1)</td>
</tr>
<tr>
<td>rs11225297</td>
<td>3' FR² A/T</td>
<td>20.7%</td>
<td>0.629</td>
<td>1.0 (0.8-1.2)</td>
</tr>
</tbody>
</table>

Abbreviations: OR, odds ratio; CI, confidence interval.

*Major and minor alleles as determined by the distribution among SECS controls.

†Minor Allele Frequency (MAF) among SECS controls.

‡Odds ratio and 95% confidence interval for the risk of endometrial cancer, age and education adjusted; AA, major allele homozygous; BB, minor allele homozygous, AB heterozygous; \( P \) value for trend.
5A/6A (rs35068180 and rs3025059) and endometriosis found mixed results. No association for either SNP was seen among 56 cases and 71 controls (31), whereas the MMP-1 2G allele was found to confer an increased risk of endometriosis among 100 cases and 150 controls (32). Similarly, the MMP-1 -1607 2G allele was found to confer an increased risk of endometrial adenocarcinoma among 100 cases and 150 controls (33), whereas no difference was seen between 107 cases and 213 controls (34). Unfortunately, neither of these functional SNPs were genotyped in the current study. However, neither of these functional promoter SNPs were genotyped. Although MMP-7 rs675620 was genotyped and shares moderate linkage disequilibrium with MMP-1 rs1799750 (D = 0.79; r² = 0.60; ref. 35); no association with endometrial cancer risk was observed. To our knowledge, no previous studies of endometrial cancer risk have been conducted. In this study, both of the functional promoter MMP-7 SNPs were genotyped. Although MMP-7 -153 C/T (rs11568819) was not found to be polymorphic in this population, MMP-7 -181 A/G (rs11568818) and another promoter SNP in high linkage disequilibrium (rs17098318; D = 1.0; r² = 0.99) both seemed to confer an increased risk of endometrial cancer in homozygote carriers of the rare allele. This is similar to our findings for breast cancer risk among premenopausal women (17), and may indicate a real, but low prevalence association that the current study lacked adequate power to detect under recessive models. Given the size of our study population, this analysis had only 31% power to detect a recessive effect of an odds ratio of 2.0 for a SNP with a MAF of 10%, >93% power to detect an odds ratio of 1.3 for a SNP with a MAF of 20%, and >77% power to detect an odds ratio of 1.2 for a SNP with a MAF of 30%. In summary, 30 haplotype tagging polymorphisms in MMP-1, MMP-3, and MMP-7 were evaluated among 1,037 endometrial cancer cases and 1,018 controls; none were found to be significantly associated with endometrial cancer risk.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
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References
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