Polymorphisms in MMP-2 and MMP-9 promoter regions are associated with endometriosis

In this case-control study, we investigated the potential associations of MMP-2 and MMP-9 gene promoter region polymorphisms as well as MMP-2 promoter haplotypes with susceptibility to endometriosis in women of caucasian origin. The results demonstrated that polymorphisms in MMP-2 (−735 C/T) and MMP-9 (−1562 C/T) were associated with elevated risk of endometriosis and that certain MMP-2 promoter haplotypes were more common in control group. (Fertil Steril 2010;94:1560–3. ©2010 by American Society for Reproductive Medicine.)

Endometriosis is a multifactorial and polygenic gynecologic disorder that can have a negative impact on a woman’s health and reproductive potential. Although endometriosis occurs in 6%–10% of women of reproductive age, the prevalence among infertile patients is 2–5 times higher (1). Sampson’s implantation theory of women of reproductive age, the prevalence among infertile patients is 2–5 times higher (1). Sampson’s implantation theory of the origin of endometriosis (2) postulates that during menstruation, endometrial cells reflux into the abdomen and form ectopic endometriotic lesions. This process involves the physiologic processes of adhesion, proliferation, and angiogenesis. Additionally, complete remodeling of the extracellular matrix (ECM) is necessary for the ectopic growth of endometrial implants, which requires the presence of matrix metalloproteinases (MMPs). Endometrial stromal cells express several MMPs, including MMP-2 and MMP-9, which seem to play a key role in endometrial ECM breakdown. Altered expression of these proteinases may lead to the establishment and progression of endometriosis, because aberrant MMP-2 and MMP-9 gene expression has been reported in eutopic and ectopic endometrial tissue of endometriosis patients (3–6).

Earlier studies have shown that some single-nucleotide polymorphisms (SNPs) in the promoter regions of MMP-2 and MMP-9 lead to changes in gene expression levels and thus could be associated with predisposition to a variety of diseases (7–9). Four promoter SNPs (−735 C/T, −790 T/G, −1306 C/T, and −1575 G/A) have been described for MMP-2. The minor alleles of the −735 C/T and −1306 C/T SNPs were found to cause diminished promoter activity due to disruption of the transcription factor Sp1 binding element (7, 10). Also, the transcriptional activity of MMP-9 is reportedly influenced by the −1562 C/T SNP within the promoter region (9). Therefore, it is reasonable to assume that genetic variations in MMP-2 and MMP-9 promoter regions contribute to the predisposition to endometriosis. The aim of the present case-control study was to investigate associations between the risk of developing endometriosis and three SNPs (−735 C/T, −790 T/G, and −1575 G/A) in the MMP-2 promoter region and one SNP (−1562 C/T) in the MMP-9 promoter region, as well as to describe MMP-2 promoter haplotypes in women of caucasian origin.

A total of 150 women (18–45 years old) with surgically and histologically confirmed endometriosis were recruited into the study from Tartu University Hospital’s Women’s Clinic and Nova Vita Clinic between February 2005 and February 2008. Disease stages according to the American Society for Reproductive Medicine revised classification system (11) were as follows: stage I-II: 92 patients; stage III-IV: 58 patients.

Peripheral blood samples and a questionnaire of clinical data were collected from all participants. One hundred ninety-nine healthy women (30–50 years old) with proven fertility (at least
TABLE 1

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Control subjects</th>
<th>All patients</th>
<th>Patients with stage I-II endometriosis</th>
<th>Patients with stage III-IV endometriosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
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<tr>
<td></td>
<td>P value</td>
<td>P value</td>
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<td>P value</td>
</tr>
<tr>
<td>MMP-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>142 (71.4)</td>
<td>124 (82.7)</td>
<td>142 (71.4)</td>
<td>124 (82.7)</td>
</tr>
<tr>
<td></td>
<td>26 (14.3)</td>
<td>57 (36.8)</td>
<td>26 (14.3)</td>
<td>57 (36.8)</td>
</tr>
<tr>
<td>T/T</td>
<td>2 (1.5)</td>
<td>6 (4.0)</td>
<td>2 (1.5)</td>
<td>6 (4.0)</td>
</tr>
<tr>
<td></td>
<td>3 (2.2)</td>
<td>5 (3.3)</td>
<td>3 (2.2)</td>
<td>5 (3.3)</td>
</tr>
<tr>
<td>T/C</td>
<td>60 (31.3)</td>
<td>70 (47.2)</td>
<td>60 (31.3)</td>
<td>70 (47.2)</td>
</tr>
<tr>
<td></td>
<td>47 (79.0)</td>
<td>56 (80.0)</td>
<td>47 (79.0)</td>
<td>56 (80.0)</td>
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<tr>
<td></td>
<td>3 (5.0)</td>
<td>3 (4.2)</td>
<td>3 (5.0)</td>
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<td>2 (7.4)</td>
<td>2 (2.8)</td>
<td>2 (7.4)</td>
<td>2 (2.8)</td>
</tr>
</tbody>
</table>

Note: The number of patients was compared with the number of control subjects. Odds ratio was calculated using logistic regression analysis adjusted for height and smoking status (nonsmokers vs. smokers).

CI = confidence interval; NS = not significant; OR = odds ratio.


The comparison of general characteristics revealed that the mean ± SD age was 32.1 ± 6.1 years for the endometriosis group and 39.8 ± 5.3 years for the control group (P<.0001). Women with endometriosis were taller than control subjects (167.2 ± 5.7 cm vs. 165.4 ± 5.4 cm, respectively; OR 1.06 for 1 cm increase, 95% CI 1.02–1.11; P=.003). They were also more likely to be nonsmokers compared with women in the control group (86.0% vs. 69.2%, respectively; active smoking vs. nonsmoking OR 0.37, 95% CI 0.21–0.63; P<.001).

We determined the genotype frequencies for the MMP-2 −735 C/T, −790 T/G, and −1575 G/A and MMP-9 −1562 C/T polymorphisms. We found no significant differences in genotype frequencies of the MMP-2 −790 T/G and the −1575 G/A SNP between the patients and control subjects (data not shown). Logistic regression analysis showed that individuals with MMP-2 −735 CC genotype had almost twice the risk of endometriosis compared with women with TT or TC genotype (Table 1). By dividing patients according to the disease severity, we observed that women with MMP-2 −735 CC genotype had an elevated chance of developing stage I-II endometriosis compared with women with the TT or TC genotype (Table 1). The effect of the CC genotype on the risk of stage III-IV disease did not reach statistical significance.

All of the studied SNPs from the MMP-2 promoter region were in strong linkage disequilibrium (LD) and formed a single haplotype block. Eight haplotypes were defined for the MMP-2 promoter region; three of them (HAP1–HAP3) occurred in 94.6% of subjects. The frequency of the most common haplotype, HAP3 (−735 C, −790 T, and −1575 G alleles), was significantly higher (P=.02) among women with endometriosis (65.8%) than control subjects (56.9%), but after a permutation test (n = 1000) it no longer reached statistical significance. The occurrences of HAP4 (−735 C, −790 G, and −1575 G alleles) and HAP5 (−735 C, −790 T, and −1575 A alleles) in the endometriosis group were 0.1% for both haplotypes and were significantly higher (3.4% and 3.3% for HAP4 and HAP5, respectively) in the control group (P<.01). The statistical significance of HAP4 and HAP5 remained relevant also after a permutation test (P=.01 for both haplotypes).
The distribution of MMP-9 –1562 C/T genotypes was similar in endometriosis patients and control subjects (Table 1). However, after classifying patients into subgroups according to the disease severity, the MMP-9 –1562 TT genotype increased the odds ratio of stage III-IV endometriosis almost eightfold relative to the CC genotype (Table 1). When the TT and TC genotypes were combined, the risk of stage III-IV endometriosis was still two times higher than that in women with the major CC genotype.

Only one earlier study has investigated the involvement of MMP-2 –735 C/T SNP in the development of endometriosis (14). In a population of women from North China, Kang et al. (14) did not observe any significant association between this variation and endometriosis. In contrast, we demonstrated that in a population of caucasian women, the CC genotype of the MMP-2 –735 C/T SNP results in almost twice the risk for endometriosis compared with women having either the TT or the TC genotype. This genotype was also associated with an elevated risk of stage I-II endometriosis.

Although the relationship between MMP-2 –735 C/T and endometriosis is only beginning to be defined, earlier in vivo and in vitro studies have associated the MMP-2 –735 C allele with increased promoter activity and susceptibility to certain cancers and heart failure (7, 15, 16). These studies along with the present results suggest that the MMP-2 –735 CC genotype may increase the risk of endometriosis owing to locally higher MMP-2 transcriptional and enzymatic activities facilitating the invasion and survival of endometriotic implants in ectopic locations. The other two MMP-2 SNPs, –790 T/G and –1575 G/A, are neutral SNPs that have little to no effect on the promoter’s activity (10), and, based on our results, they are not relevant to endometriosis risk. Similar to three earlier studies (7, 14, 17), we also observed significant LD between all three SNPs studied in the MMP-2 promoter region. We identified three main MMP-2 haplotypes accounting for >90% of all haplotypes studied. The major haplotype, HAP1, was more common in patients, whereas haplotypes HAP4 and HAP5 were more frequent in control subjects. Similar findings were reported by Vasku et al. (17) in their study of four MMP-2 polymorphisms (–735 C/T, –790 T/G, –1306 C/T, and –1575 G/A), in which a significant association was found between the HAP1 haplotype and an elevated risk of coronary triple-vessel disease. Furthermore, Vasku et al. (17) identified two haplotypes similar to our HAP4 and HAP5 which were absent in patients but present in the control group. Because the HAP4 and HAP5 haplotypes were more prevalent among control subjects in the present study, we can assume that the presence of these haplotypes may protect women from developing endometriosis.

In vitro expression studies on the MMP-9 –1562 C/T polymorphism have confirmed that the allelic change of C to T results in loss of a binding site for a transcription repressor protein, resulting in a doubling of promoter activity (9). In the present study, we found that the distribution of MMP-9 –1562 C/T genotypes were similar across both groups of women. However, when patients were divided into subgroups according to the disease severity, a significant association was observed between the occurrence of the TT or TC genotype and stage III-IV endometriosis. Therefore we hypothesize that the presence of T allele in MMP-9 –1562 C/T polymorphism could indicate the development of only severe forms of endometriosis or create a precondition for progression of minimal-mild to moderate-severe form of endometriosis. However, this finding conflicts with two earlier studies that showed no significant relationship between the –1562 C/T SNP and stage III-IV endometriosis in Asian women (18, 19). Still, discrepancies between the studies could be due to population differences.

The interpretation of the present study results is somewhat limited, because we included only fertile women with ≥ 2 children and no medical history of endometriosis in the control group. Therefore, we cannot entirely exclude the presence of undiagnosed asymptomatic endometriosis patients among the control women. However, we believe that the inclusion of undiagnosed cases could lead more likely to false-negative than false-positive associations, with a marginal dilution of the effects of the risk factors.

In conclusion, the present case-control study demonstrated that individual variations in MMP-2 and MMP-9 promoter regions as well as certain MMP-2 promoter haplotypes may influence the susceptibility to endometriosis in a caucasian population.

REFERENCES


