Polymorphisms in the promoter regions of the matrix metalloproteinases-7, -9 and the risk of endometriosis and adenomyosis in China

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Matrix metalloproteinases (MMPs) may contribute to the development of endometriosis. The aim of this study was to assess the effects of the polymorphisms in the promoters of MMP-7 (181A/G) and MMP-9 (1562C/T) on the risk of occurrence of endometriosis and adenomyosis. We genotyped 219 patients (143 women with endometriosis, 76 women with adenomyosis) and 160 control women in North China. There was a significant difference in frequency of the MMP-7 genotype between endometriosis and controls (p = 0.01) and also between adenomyosis and controls (p = 0.01). The frequency of the G allele in two groups of patients (7.3 and 7.9%) was significantly higher than in the controls (2.8%) (p = 0.01 and 0.01, respectively). Compared to the A/A genotype, the genotype with the -181G allele showed a significantly increased susceptibility to both diseases, with adjusted odds ratio of 2.62 [95% confidence interval (CI) = 1.17–5.87] for endometriosis and 3.14 (95% CI = 1.26–7.81) for adenomyosis. However, the overall genotype and allelotype distribution of the -181C/T polymorphism may not provide a useful marker to predict susceptibility to endometriosis and adenomyosis, at least in women from North China.

Key words: adenomyosis/endometriosis/matrix metalloproteinase-7/matrix metalloproteinase-9/single nucleotide polymorphism

Introduction
Endometriosis is a common gynaecological disease, characterized histologically by the presence of endometrial glands and stroma outside the uterine cavity, which plays an important role in female infertility. Adenomyosis has some similarities to endometriosis, being characterized by the presence of ectopic endometrium within the myometrium. The two diseases are invasive but benign diseases that require remodelling of the extracellular matrix (ECM) for ectopic implantation of endometrium. Matrix metalloproteinases (MMPs) are an important group of zinc enzymes that are responsible for degradation of ECM components such as collagen and proteoglycans in normal embryogenesis and remodelling and in many disease processes such as carcinoma invasion. The over-expression of MMPs has been demonstrated in a variety of cancers and is correlated with invasion and tumour metastasis, for example in oral cancer (Jordan et al., 2004), colorectal cancer (Adachi et al., 1999), esophageal squamous cell carcinomas (Ohashi et al., 2000) and endometrial cancer (Aglund et al., 2004). Like carcinoma, endometriosis and adenomyosis have the unique characteristics of invasion and metastasis, though pathologically, they are benign. Previous studies have shown that many MMPs were more highly expressed in ectopic endometrium than in eutopic endometrium in women with endometriosis. Thus the over-expression of MMPs may contribute to the development of endometriosis (Kokorine et al., 1997; Cox et al., 2001; Ssamatowicz et al., 2002; Gilabert-Estelles et al., 2003).

There are several regulating mechanisms that may influence the activities of MMPs such as regulation of transcription, activation of latent MMPs, inhibition of MMP function by tissue inhibitors of metalloproteinases (TIMPs) and regulation by ovarian steroid hormones and so on. Of these mechanisms, the most important may be transcriptional regulation; since most MMPs genes are expressed only when physiologically active or pathological tissue remodelling takes place. There is growing evidence to indicate that natural sequence variations in promoters of the MMPs genes may result in different expression of MMPs in different individuals (Ye, 2000). These polymorphisms have been associated with susceptibility to some diseases including acute myocardial infarction (Nojiri et al., 2003), rheumatoid arthritis (Mattey et al., 2004), multiple sclerosis (Fiotti et al., 2004) and cancers (Yu et al., 2002; Zhang et al., 2004; Fang et al., 2005).

We hypothesize that increased expression of MMPs gene polymorphisms might promote the susceptibility to endometriosis and adenomyosis. We have previously studied the association between promoter polymorphisms in MMP-1 and MMP-3 genes and patient susceptibility to endometriosis (Kang et al., 2005) and found that the MMP-1 promoter single nucleotide polymorphism (SNP) and the MMP-2G6A haplotype may modify susceptibility to endometriosis. In the present study, we investigated the role of MMP-7 (181A/G) and MMP-9 (1562C/T) in the development of endometriosis and adenomyosis to test the above hypothesis.
Materials and methods

Study participants
Blood was obtained from the following three groups and DNA was extracted for genotyping: (i) healthy female blood donors aged 25–50 years (n = 160) and (ii) the endometriosis cases were inpatients for endometriosis in the Fourth Hospital, Hebei Medical University between 2001 and 2004 (n = 143). The patients were all clinically, endoscopically and histologically confirmed. None of the endometriosis patients had taken any hormonal treatment. All patients were in endometriosis stages III and IV. Patients were staged according to the revised American Fertility Society (AFS, 1985) classification. (iii) The adenomyosis cases were inpatients for adenomyosis in the same hospital between 2002 and 2004 (n = 76). None of these patients had taken any hormonal treatment. General information of all patients was recorded in detail in the medical records. The endometriosis patients who also had adenomyosis were excluded from the study.

The control group consisted of women of reproductive age without any malignant disease confirmed by surgical exploration at voluntary abortion, cesarean section or pathologically confirmed after hysterecomy performed for dysfunctional uterine bleeding. The general information of the healthy controls was extracted from their medical records. The study was approved by the Ethics Committee of Hebei Obstetrics and Gynecology Institute and informed consent was obtained from all recruited subjects.

DNA extraction
Venous blood (5 ml) was drawn from each subject into Vacutainer tubes containing EDTA and stored at 4°C. Genomic DNA was extracted within one week after sampling using proteinase K (Merck, Darmstadt, Germany) digestion followed by a salting out procedure according to the method of Miller et al. (1988).

MMP-7 SNP genotyping
The MMP-7–181A/G genotypes were determined by PCR–RFLP assay. The primers for amplifying the MMP-7 fragment were 5′-TGGTACCATAATGTCCGATG-3′ (forward) and 5′-TCGTTATGGCCAGGACACACAGTTAATG-3′ (reverse) (Jormajo et al., 2001). The PCR was performed in a 20 μl volume containing 100 ng of DNA template, 2.0 μl of 10× PCR buffer, 1.5 mM of MgCl₂, 1 U of Taq-DNA-polymerase (BioDev-Tech), 200 μM of dNTPs and 200 nM of each primer. The PCR cycling conditions were 5 min at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at 65°C and 30 s at 72°C and with a final step at 72°C for 5 min to allow for the complete extension of all PCR fragments. An 8 μl aliquot of PCR product was subjected to digestion at 37°C overnight in a 10 μl reaction containing 10 U of EcoR I (TakaRa Biotechnology Co. Ltd, Dalian, China) and 1× reaction buffer. After digestion, the products were separated on a 4% agarose gel stained with ethidium bromide. The MMP-7–1562 T allele is digested by SpI, yielding fragments of 247 and 188 bp while the -1562 C allele is not cleaved by SpI and keeps the original PCR product (435 bp), the heterozygotes displayed a combination of both alleles (435, 247 and 188 bp) (Figure 2).

For a negative control, distilled water was used instead of DNA in the reaction system for each panel of PCR. The PCR reactions of 10% of the samples were run in duplicate for quality control.

Statistical analysis
Statistical analysis was performed using the SPSS10.0 software package (SPSS Company, Chicago, IL, USA). Hardy–Weinberg analysis was performed to compare the observed and expected genotype frequencies using the chi-square test. Comparison of the MMP-7 and MMP-9 genotype distribution in the study groups was performed by means of two-sided contingency tables using chi-square test. The odds ratio (OR) and 95% confidence interval (CI) were calculated using an unconditional logistic regression model. A probability level of 5% was considered significant.

Results
Association of MMP-7 SNP with susceptibility to endometriosis and adenomyosis
The distribution of the MMP-7 genotypes in controls did not significantly deviate from that expected for a Hardy–Weinberg equilibrium (χ² = 0.00, P = 1.00). The frequency of the G allele among endometriosis and adenomyosis patients (7.3 and 7.9%) was significantly higher than in the healthy controls (2.8%) (χ² = 6.59 and 6.26, P = 0.01 and 0.01, respectively). The frequencies of the A/A and A/G + G/G genotypes in endometriosis and adenomyosis patients were significantly different from those in control (χ² = 6.10 and 6.57, P = 0.01 and 0.01, Table I). Compared with the A/A genotype, the A/G + G/G genotype significantly modified the risk of developing endometriosis and adenomyosis. The odds ratios were 2.62 (95% CI = 1.17–5.87) and 3.14 (95% CI = 1.26–7.81) (Table II).

![Figure 1. MMP-7 genotyping by PCR–RFLP analysis followed by separation on 4% agarose gel as described in text. Lane 1, 100 bp ladder; lanes 2 and 3, A/A; lanes 4 and 5= A/G; lane 6, G/G.](https://academic.oup.com/molehr/article-abstract/12/1/35/1045983)

![Figure 2. MMP-9 genotyping by PCR–RFLP analysis followed by separation on 2% agarose gel as described in text. Lane 1, 100 bp ladder; lanes 2 and 6, C/C; lanes 3 and 5, C/C; lanes 4 and 7, T/T.](https://academic.oup.com/molehr/article-abstract/12/1/35/1045983)
Table I. Distribution of genotypes and alleles of a single nucleotide polymorphism in MMP-7 and MMP-9 genes

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control [n = 160 (%)]</th>
<th>Endometriosis [n = 143 (%)]</th>
<th>P value</th>
<th>Adenomyosis [n = 76 (%)]</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>MMP-7 genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>151 (94.4)</td>
<td>123 (86.0)</td>
<td>0.01</td>
<td>64 (84.2)</td>
<td>0.01</td>
</tr>
<tr>
<td>AG</td>
<td>9 (5.6)</td>
<td>19 (13.3)</td>
<td></td>
<td>12 (15.8)</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>0 (0)</td>
<td>1 (0.7)</td>
<td></td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>MMP-7 allelotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>311 (97.2)</td>
<td>265 (92.7)</td>
<td>0.01</td>
<td>140 (92.1)</td>
<td>0.01</td>
</tr>
<tr>
<td>G</td>
<td>9 (2.8)</td>
<td>21 (7.3)</td>
<td></td>
<td>12 (7.9)</td>
<td></td>
</tr>
<tr>
<td>MMP-9 genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>134 (83.8)</td>
<td>112 (78.3)</td>
<td>0.23</td>
<td>60 (79.0)</td>
<td>0.37</td>
</tr>
<tr>
<td>C/T</td>
<td>26 (16.2)</td>
<td>30 (21.1)</td>
<td></td>
<td>15 (19.7)</td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>0 (0)</td>
<td>1 (0.7)</td>
<td></td>
<td>1 (1.3)</td>
<td></td>
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<tr>
<td>MMP-9 allelotype</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>294 (91.9)</td>
<td>254 (88.8)</td>
<td>0.20</td>
<td>136 (89.5)</td>
<td>0.39</td>
</tr>
<tr>
<td>T</td>
<td>26 (8.1)</td>
<td>32 (11.2)</td>
<td></td>
<td>16 (10.5)</td>
<td></td>
</tr>
</tbody>
</table>

Association of MMP-9 SNP with susceptibility to endometriosis and adenomyosis

The distribution of the MMP-9 genotypes in controls was consistent with the Hardy–Weinberg equilibrium (χ² = 1.08, P = 0.58). The frequencies of the C allele among endometriosis, adenomyosis patients and healthy controls were 88.8, 89.5 and 91.9%, respectively. The frequencies of the T allele among endometriosis patients, adenomyosis patients and healthy controls were 11.2, 10.5 and 8.1%, respectively. No significant difference in the MMP-9 allele distribution was shown between the endometriosis, adenomyosis patients and controls (χ² = 1.64 and 0.73, P = 0.20 and 0.39, Table I). There was no significant difference in genotype (C/C and C/T + T/T) distribution between endometriosis, adenomyosis patients and healthy women (χ² = 1.46 and 0.81, P = 0.23 and 0.37). Compared with the C/C genotype, the C/T + T/T genotype did not significantly modify the risk of developing endometriosis. The adjusted odds ratio was 1.55 (95% CI = 0.89–2.72) and 1.37 (95% CI = 0.69–2.75) (Table II).

Discussion

This study shows that the MMP-7–181A/G genotype may be a potentially indicative factor for susceptibility to endometriosis and adenomyosis, that is, genotypes with the G allele significantly increase the risk of development of endometriosis and adenomyosis. However, the MMP-9–1562C/T polymorphism may not be associated with the occurrence of endometriosis and adenomyosis. To the best of our knowledge, this is the first study to look for an association between polymorphisms of MMP-7 and MMP-9 and the risk of development of endometriosis and adenomyosis.

MMP-7 (also known as matrilysin or PUMP-1) is one of the smallest members of the MMP family described to date. Unlike other MMPs, which are expressed in stromal cell, MMP-7 is an epithelial-specific MMP (Wilson and Matrisian, 1996). The over-expression of MMP-7 has been documented in several cancer types as correlating with the potential for invasive cancer (Kioi et al., 2003; Leeman et al., 2003; Ajisaka et al., 2004). In the uterus, MMP-7 mRNA has not been detected in normal, secretory-phase endometrium, but strong expression has been observed in eutopic or ectopic endometrium removed during the secretory phase from a patient with endometriosis (Bruner et al., 2002).

An A to G substitution at position -181 (-181A/G) is reported to influence the binding of nuclear proteins in the promoter region of MMP-7, and the G allele was associated with higher basal transcriptional activity (Birkedal-Hansen et al., 1993). A study of colorectal carcinoma patients suggested that the presence of the G allele in the MMP-7 gene promoter sequence may be a facilitating factor for cancer growth, lymph node invasion and metastasis (Ghilardi et al., 2003). Our findings are consistent with this, suggesting that the G allele of MMP-7 may enhance risk of endometriosis and adenomyosis. However, the G allele frequencies of controls in our study was lower than in the Italian population (2.8 versus 40.1%) (Ghilardi et al., 2003). In a previous study we found the same result that G allele of MMP-7 may be associated with esophageal squamous cell carcinoma, gastric cardiac adenocarcinoma and non-small cell lung carcinoma (NSCLC) (Zhang et al., 2005).

The increased promoter activity of the -181G allele may induce elevation of the MMP-7 mRNA and in turn increase protein expression (Jormsjo et al., 2001). MMP-7 can not only degrade elastin, proteoglycans, fibronectin and type IV collagen (Quantin et al., 1989), but also cleaves non-matrix substrates from the cell surface, including E-cadherin (Noe et al., 2001), pro-tumour necrosis factor a (TNF-a) (Haro et al., 2000) and Fas ligand (Powell et al., 1999). E-cadherin is an important cell adhesion protein that forms a key part of the adhesive junctions between epithelial cells (Gumbiner, 1996). The reduction of E-cadherin in ectopic endometrium results in decreased adhesion between the epithelial cell and stromal cell and between the endometrial epithelial cells. Consequently, it is easier for shed epithelial cells from the endometrium to relocate and form ectopic lesions (Scotti et al., 2000; Poncelet et al., 2002). Additionally, FasL has an important role in apoptosis. The over-expression of MMP-7 could result in the shedding of FasL from the cell surface and the generation of soluble FasL with less potent activity in terms of triggering apoptosis by cross-linking with Fas through the recently described TNFRSF6/
TNFSF6 [Fas/Fas ligand (Fasl)] pathway (Schneider et al., 1998; Powell et al., 1999; Poulaki et al., 2001). A less effective Fasl pathway would more readily enable cancer cells to evade apoptosis and thus promote tumour survival (Igny and Kramer, 2002). We believe that a similar mechanism may explain the survival of endometrial cells in the peritoneal cavity of women with endometriosis. MMP-9, also known as gelatinase B or 92-kDa type IV collagenase, has a broad substrate specificity, being particularly active against gelatins and type IV collagen (Birkedal-Hansen et al., 1993). It also possesses proteolytic activity against proteoglycan core protein and elastin. The MMP-9 in ectopic endometrium was significantly higher than in eutopic endometrium (Chung et al., 2001; Yu et al., 2003). This increased expression may be one of the reasons for the invasive properties of the endometrium, resulting in the development of endometriosis (Chung et al., 2001).

A transition of C to T at the 1562 base pair position upstream of the transcription initiation site (C-1562T) of MMP-9 has been reported. In vitro studies have demonstrated that the -1562T MMP-9 alleles are associated with increased promoter activity. Transient transfection experiments showed that the -1562T MMP-9 allele had a two-fold higher promoter activity than the -1562C allele (Zhang et al., 1999). Matsumura (Matsumura et al., 2005) reported that the T allele of the MMP-9 polymorphism is linked with the invasive phenotype of gastric cancer, but, Grieu and others (2004) thought that the MMP-9–1562T allele was associated with a better prognosis than C homozygotes in breast cancer. Our study suggests that the MMP-9C-1562T polymorphism is not associated with susceptibility for endometriosis and adenomyosis. This is consistent with our study into NSCLC (Wang et al., 2005). This discrepancy may be explained first by the different role the MMP-9 polymorphism plays in different diseases. Second, although it has been shown that the MMP-9–1562T allele has a higher promoter activity than the C allele, which may be because of the preferential binding of a putative transcription repressor protein to the C allelic promoter (Pollanen et al., 2001), the significant difference in promoter activity between the -1562C and -1562T alleles has not been observed in primary amnion epithelial cell cultures (Ferrand et al., 2002). Therefore, the significance of the MMP-9 C-1562T polymorphism in disease development and progression should be further investigated in the different genotypes and phenotypes. Finally, further studies on other functional polymorphisms in the MMP-9 gene may help to explore the role of MMP-9 in development of disease.

So far, the aetiology of endometriosis and adenomyosis is unknown. Although adenomyosis and endometriosis differ with respect to diagnosis and treatment, these two diseases seem to share a common developmental mechanism with respect to MMP.

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Polymorphisms in the promoter regions


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