Estrogen receptor α-351 XbaI*G and -397 PvuII*C-related genotypes and alleles are associated with higher susceptibilities of endometriosis and leiomyoma

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Endometriosis and leiomyoma are both common estrogen-related gynaecological diseases. We aimed to elucidate the association of estrogen receptor α (ERα)-351 A>G (XbaI) and -397 T>C (PvuII) gene polymorphisms with endometriosis and leiomyoma. Women were divided into three groups: (i) severe endometriosis (n = 112), (ii) leiomyoma (n = 106) and (iii) normal controls (n = 110). Genomic DNA was obtained from peripheral leukocytes. ERα-351 A/G XbaI and -397 T/C PvuII polymorphisms were assayed by the method of PCR and restriction fragment length polymorphism (RFLP). Genotypes and allelic frequencies in each group were compared. The genotype/allele frequencies of ERα-351 and -397 polymorphisms in endometriosis or leiomyoma groups were different from those of normal controls. ERα mutant-related genotypes/alleles (-351G and -397C) presented higher percentages in the endometriosis/leiomyoma population compared with normal controls. Proportions of ERα-351 AA/AG/GG genotypes and A/G alleles in each group were (i) 26.8/57.1/16.1 and 55.4/44.6%; (ii) 19.8/52.8/27.4 and 46.2/53.8% and (iii) 33.6/54.5/12.9% and 64.6/1.8 and 65.9/34.1%. Proportions of ERα-351 A/G and -397 T/C allele frequencies in each group were compared. The genotype/allele frequencies of ERα-351 and -397 polymorphisms in endometriosis or leiomyoma groups were different from those of normal controls. ERα mutant-related genotypes/alleles (-351G and -397C) presented higher percentages in the endometriosis/leiomyoma population compared with normal controls. Proportions of ERα-351 AA/AG/GG genotypes and A/G alleles in each group were (i) 26.8/57.1/16.1 and 55.4/44.6%; (ii) 19.8/52.8/27.4 and 46.2/53.8% and (iii) 33.6/64.6/1.8 and 65.9/34.1%. Proportions of ERα-397 TT/TC/CC genotypes and T/C alleles in each group were (i) 24.1/60.7/15.2 and 54.5/45.5%; (ii) 23.6/70.8/5.6 and 59/41% and (iii) 54.5/40/5.5 and 74.5/25.5%. We concluded that ERα-351 XbaI*G- and -397 PvuII*C-related genotypes/alleles were correlated with higher susceptibilities of endometriosis or leiomyoma, which might be associated with related pathogeneses.

Key words: endometriosis/estrogen receptor/leiomyoma/polymorphism/single-nucleotide polymorphism

Introduction

Endometriosis, a common gynaecological disorder in premenopausal women, occurs in around 10% of the female population (Goldman and Cramer, 1989) and as high as 30–40% in infertile women (Strathy et al., 1982). Endometriosis is a polygenic/multifactorial disease, which is related to the complex interactions between hormone and cytokine activation, immunoinflammatory processes, genetic factors and the environment (Kennedy, 1998). Three possible theories have been proposed for endometriosis, including (i) retrograde menstruation through the fallopian tubes into the peritoneal cavity, (ii) lymphatic and vascular metastases and (iii) tissue in situ metaplasia. Numerous factors have been implicated in the formation of ectopic endometrium implants, including the ovarian hormones, estrogen and progesterone (Irahara et al., 2001). Estrogen secreted by the ovaries is necessary for the development of endometriosis. Endometriosis develops mostly in women of reproductive age and regresses after menopause or ovariectomy, which suggests estrogen-dependent growth. Ectopic endometrium persistently expresses estrogen receptor (ER), independently of the menstrual phase. Furthermore, the biological activity of the activated receptor in ectopic implants is thought to differ from that in eutopic endometrium (Nisolle et al., 1997).

Leiomyoma, the most common neoplasm of uterus, occurs in around one-fourth of women during their lifetime (Cramer, 1992). Despite its high prevalence, the related pathophysiology and proliferative pathway remains obscure. One possible mechanism is the different expression of estrogen-regulated genes between leiomyoma and normal myometrium (Andersen and Barbieri, 1995). Leiomyoma tissue appears more sensitive to estrogen than myometrium. Myoma growth is regulated not only by serum estrogen but also by estrogen in the tumour itself (Urabe et al., 1990). Tissue concentration of estrogen is higher in leiomyoma tissues than in normal myometrium (Urabe et al., 1990). In addition to sexual hormones, the pathogenesis of leiomyoma involves multiple local growth factors, acting in an autocrine or paracrine fashion (El-Badry et al., 1991). Although the pathogenesis of endometriosis or leiomyoma remains unclear, both gynaecological diseases are known to be estrogen-dependent and have a genetic component. The effects of estrogens are mediated primarily through ER in endometriosis or leiomyoma tissues.

Heritable genetic factors may contribute to the initiation and progression of endometriosis or leiomyoma (Treloar et al., 1999). Gene polymorphisms are useful tools in the study of multifactorial disorders (Anderson et al., 1994). Polymorphisms involved in steroid hormone biosynthesis and signalling may be useful genetic biomarkers for hormone-related diseases (Dunning et al., 1999). Molecular geneticists are developing the third-generation human genome map with single-nucleotide polymorphisms (SNPs). Genetic studies of multifactorial
disease such as endometriosis or leiomyoma are difficult because of the uncertainty of the polygenic trait. The identification of the related genes is essential for genetic diagnosis and gene therapy for genetic-associated disease. The analyses of SNPs can be implemented to analyze the mechanisms of complex genetic disorders.

ER is also involved in metabolic pathways influencing estrogen-related tissue growth and height stature (Schnit et al., 2004a). ERα and ERβ mediate much estrogen action. ER is a member of the nuclear receptor superfAMILY of ligand-activated transcription factors, which mediates estrogen actions in target tissues. Different polymorphisms have been described in ERα genes. Allolic variants of the gene encoding ERα and ERβ may alter their expression and function, resulting in genetic variability. Several polymorphisms of ERα gene have been reported to be associated with alterations in receptor expression and function. The ERα gene, which is located on chromosome 6q25, contains some gene polymorphisms, including intron 1 polymorphisms XbaI (dbSNP: rs93407099) and PvuII (dbSNP: rs2234693) (Ioannidis et al., 2004; van Duijnhoven et al., 2005). The associations between the ERα polymorphism and breast cancer or osteoporosis have been demonstrated (Liu et al., 2001; Boyapati et al., 2005).

Despite many epidemiological studies suggested that the ERα genetic variants confer increased susceptibility to individual disorders, few investigators demonstrated their association with endometriosis or leiomyoma. Reviewing the MEDLINE database, only two reports presented the non-association of ERα XbaI and PvuII polymorphisms with the susceptibility of endometriosis (Wang et al., 2004; Kim et al., 2005). Furthermore, no investigators demonstrated correlations with leiomyoma. In our previous articles, we observed the correlation of endometriosis or leiomyoma with some hormone-related SNPs, including ER thymine–adenine (TA) dinucleotide repeat polymorphism (Hsieh et al., 2003, 2005a), progesterone receptor Alu insertion (Hsieh et al., 2005b) and androgen receptor trinucleotide polymorphism (Hsieh et al., 2001, 2004). Herein, we tried to evaluate the distributions of ERα PvuII and XbaI polymorphism in Taiwanese women with endometriosis or leiomyoma.

Materials and methods

Premenopausal Taiwanese women with surgically diagnosed severe endometriosis [Revised American Fertility Society (AFC) classification of endometriosis, 1985], leiomyoma and normal individuals without endometriosis and leiomyoma were recruited. All operations were performed by two surgeons (Y.-Y.H. and C.C.C.). Patients were divided into three groups: (i) severe endometriosis (n = 112); (ii) leiomyoma (n = 106) and (iii) normal controls (n = 110). The normal controls were recruited during annual health examinations. The non-endometriosis or non-leiomyoma status was confirmed after detailed ultrasonography examination. The age of the patients in the three groups was comparable (34.2 ± 3.8 versus 35.2 ± 4.1 versus 37.1 ± 4.8 years, respectively). This article was approved by the ethical committee and institutional review board of the China Medical University Hospital. Informed consents were signed by all women who donated their blood. All women accepted the peripheral blood sampling for genotype analyses.

The ERα gene polymorphisms were determined according to previously described methods (Kobayashi et al., 1996; Lorentzon et al., 1999). The ERα-351 XbaI A/G (uncutable/cutable) and -397 PvuII T/C (uncutable/cutable) polymorphisms were assayed by the method of PCR and restriction fragment length polymorphism (RFLP). Genomic DNA was extracted from peripheral blood using Genomaker DNA extractor kit (Blossom, Taipei, Taiwan) and subjected to PCR, digestion with restriction enzymes and gel electrophoresis of the PCR products. Approximately 50 ng of genomic DNA was mixed with 20 pmol of PCR primer in a total volume of 25 μl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM each deoxyribonucleotide triphosphate and 1 unit of Amplitaq DNA polymerase (Perkin Elmer Applied Biosystems, Foster City, CA, USA). The PCR amplification was performed in a programmable thermal cycler GenAmp PCR system 2400 (Perkin Elmer Applied Biosystems). A 1374-bp fragment product, a part of intron 1 and exon 2 of ERα gene, was amplified by PCR (Kobayashi et al., 1996; Lorentzon et al., 1999). After PCR amplification, two ERα gene polymorphisms were analysed by restriction digestion with restriction enzymes (XbaI and PvuII; New England Biolabs, Inc., Beverly, MA, USA). The primer sequences, PCR condition, base pairs for their wild and mutant types after RFLP are summarized in Table I. The SNP information for the genes involved was obtained through NCBI (http://www.ncbi.nlm.nih.gov/LocusLink/).

A 5-μl PCR product was loaded into 1% agarose gel containing ethidium bromide for electrophoresis. Genotypes for XbaI and PvuII polymorphisms were termed AA/AG/GG and TT/TC/CC, respectively. Genotypes and allelic frequencies for ERα XbaI A/G and PvuII T/C gene polymorphisms in each group were compared. Correlations of ERα XbaI A/G and PvuII T/C genotypes and endometriosis/leiomyoma were evaluated. Allelic frequencies are expressed as a percentage of the total number of alleles. The SAS system (version 8.1, SAS Institute Inc., Cary, NC, USA) with χ² test was used for statistical analyses. A P-value <0.05 was considered statistically significant.

Results

Genotype distribution and allele frequency of ERα-351 XbaI A/G and -397 PvuII T/C gene polymorphisms between endometriosis/leiomyoma groups and normal controls were significantly different (Tables II–V). Genetic variations in the ERα (XbaI*G, PvuII*C) were more prevalent in the disorder groups. Higher percentages of ERα mutant genotypes/alleles (XbaI*G, PvuII*G) and wild genotypes/alleles (XbaI*G, PvuII*G) were present in the endometriosis/leiomyoma group compared with normal controls. There was no statistically significant difference between the endometriosis and leiomyoma groups in the distributions of ERα XbaI and PvuII polymorphisms. The most common genotypes and allele for ERα XbaI gene polymorphisms in group were A-related genotypes and allele. ERα XbaI*G-related genotype and allele were associated

<p>| Table I. The primer sequences, PCR and restriction fragment length polymorphism (RFLP) conditions for estrogen receptor α (ERα)-351 A/G XbaI and -397 T/C PvuII gene polymorphisms |
|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Primers sequences (5′→3′)</th>
<th>Denature (°C)</th>
<th>Annealing (°C)</th>
<th>Extension (°C)</th>
<th>Restriction enzyme (°C/min)</th>
<th>SNP sequence</th>
<th>Allelic variants</th>
<th>DNA fragment size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERα-351 A/G XbaI polymorphism</td>
<td>F-CTGCCACCCCTATCTGTATCTTTTCTATTCTCC</td>
<td>94/30</td>
<td>58/30</td>
<td>72/30</td>
<td>XbaI (37/60)</td>
<td>A (wild)</td>
<td>A</td>
<td>1374</td>
</tr>
<tr>
<td>ERα-397 T/C PvuII polymorphism</td>
<td>R-CTCTTTCTCTGCCACCCTTGGGCCTGATTATCTGA</td>
<td>94/30</td>
<td>58/30</td>
<td>72/30</td>
<td>PvuII (65/60)</td>
<td>G (mutant)</td>
<td>T</td>
<td>982 + 392</td>
</tr>
<tr>
<td>SNP, single-nucleotide polymorphism.</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

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Table II. Genotype distributions of estrogen receptor (ER)-351 A/G XbaI gene polymorphisms in women with endometriosis, leiomyoma and normal controls

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Endometriosis (n = 112)</th>
<th>Leiomyoma (n = 106)</th>
<th>Normal controls (n = 110)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA homozygote</td>
<td>30 (26.8%)</td>
<td>21 (19.8%)</td>
<td>37 (33.6%)</td>
</tr>
<tr>
<td>AG heterozygote</td>
<td>64 (57.1%)</td>
<td>56 (52.8%)</td>
<td>71 (64.6%)</td>
</tr>
<tr>
<td>GG homozygote</td>
<td>18 (16.1%)</td>
<td>29 (27.4%)</td>
<td>2 (1.8%)</td>
</tr>
</tbody>
</table>

*Not statistically different (endometriosis versus leiomyoma).

Table III. Allele frequencies of estrogen receptor (ER)-351 A/G XbaI gene polymorphisms in women with endometriosis, leiomyoma and normal controls

<table>
<thead>
<tr>
<th>Allelic variants</th>
<th>Endometriosis (n = 224)</th>
<th>Leiomyoma (n = 212)</th>
<th>Normal controls (n = 220)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A allele</td>
<td>124 (55.4%)</td>
<td>98 (46.2%)</td>
<td>145 (65.9%)</td>
</tr>
<tr>
<td>G allele</td>
<td>100 (44.6%)</td>
<td>114 (53.8%)</td>
<td>75 (34.1%)</td>
</tr>
</tbody>
</table>

A allele, wild uncuttable type; G allele, mutant cuttable type.

*Not statistically different (endometriosis versus leiomyoma).

Table IV. Genotype distributions of estrogen receptor (ER)-397 T/C PvuII gene polymorphisms in women with endometriosis, leiomyoma and normal controls

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Endometriosis (n = 112)</th>
<th>Leiomyoma (n = 106)</th>
<th>Normal controls (n = 110)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT homozygote</td>
<td>27 (24.1%)</td>
<td>25 (23.6%)</td>
<td>60 (54.5%)</td>
</tr>
<tr>
<td>TC heterozygote</td>
<td>68 (60.7%)</td>
<td>75 (70.8%)</td>
<td>44 (40.0%)</td>
</tr>
<tr>
<td>CC homozygote</td>
<td>17 (15.2%)</td>
<td>6 (5.5%)</td>
<td>6 (5.5%)</td>
</tr>
</tbody>
</table>

*Not statistically different (endometriosis versus leiomyoma).

Table V. Allele frequencies of estrogen receptor (ER)-397 T/C PvuII gene polymorphisms in women with endometriosis, leiomyoma and normal controls

<table>
<thead>
<tr>
<th>Allelic variants</th>
<th>Endometriosis (n = 224)</th>
<th>Leiomyoma (n = 212)</th>
<th>Normal controls (n = 220)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T allele</td>
<td>122 (54.5%)</td>
<td>125 (59.0%)</td>
<td>164 (74.5%)</td>
</tr>
<tr>
<td>C allele</td>
<td>102 (45.5%)</td>
<td>87 (41.0%)</td>
<td>56 (25.5%)</td>
</tr>
</tbody>
</table>

C allele, mutant cuttable type; T allele, wild uncuttable type.

*Not statistically different (endometriosis versus leiomyoma).

Discussion

Endometriosis and leiomyoma are both estrogen-dependent neoplasms of premenopausal women. Estrogen and ER play major roles in the pathogenesis of endometriosis and leiomyoma. Genetic defects and environmental factors including dietary and environmental regulating hormonal and non-hormonal conditions might contribute to the development of endometriosis and leiomyoma (Sano et al., 1995). ER-related genotypes determine the function of the sex-steroid system not only at the receptor level but also at the level of hormone synthesis (Zofkova et al., 2002).

The mechanisms of SNPs upon individual diseases remain uncertain. Unlike mutations, polymorphisms are not directly linked to certain diseases, but they are useful tools in the study of multifactorial disorders such as endometriosis and leiomyoma. Despite SNPs don’t alter transcription, the disequilibrium of genotypes might influence the related 3D structure and efficiency of the transcripts (Shintani et al., 1999; Kennon et al., 2004; Shirasawa et al., 2004). Intronic sequences have been reported to contain regulatory elements for transcription and splicing, giving rise to varying messenger RNA levels and different isoforms of mature messenger RNA, respectively (Gasch et al., 1989; Carstens et al., 1998). The ER polymorphisms might be in linkage disequilibrium with other unidentified functional gene variants, which cooperatively influence the susceptibility to endometriosis or leiomyoma.

Some polymorphic sites in the 5′ region of the ERα gene have been demonstrated. ERα-14 TA or 12/13 TA repeats are associated with a higher risk of endometriosis or leiomyoma, respectively (Hsieh et al., 2003, 2005a). The polymorphic sites defined by restriction enzymes (PvuII and XbaI) are located in the first intron of ERα gene (Kobayashi et al., 1996). ERα XbaI and PvuII gene polymorphisms have been reported to be related to numerous estrogen-related diseases (Table VI). Erα-mutant alleles (XbaI*G and PvuII*C) associated with elevated serum estradiol (E2) production (Zofkova et al., 2002; Schuit et al., 2005). Such relationships provide the molecular pathways between ERα allelic variations and endometriosis/leiomyoma pathogenesis.

In this study, we observed that the genotype distributions and allele frequencies for ERα XbaI A/G and PvuII T/C polymorphisms were significantly different between the individuals with and without endometriosis/leiomyoma. Mutant variants for both ERα SNPs are correlated with higher susceptibility to endometriosis and leiomyoma. We hypothesize that both ERα XbaI and PvuII gene polymorphisms might predisperse to endometriosis or leiomyoma development. The ERα XbaI -351*G-related genotype and allele are strongly related to the occurrence of leiomyoma, compared to being moderately correlated with the occurrence of endometriosis. In contrast, the ERα PvuII -397*C-related genetic variants are strongly correlated with endometriosis susceptibility, compared to being moderately correlated with leiomyoma risk.

In this study, we observed higher percentages of ER-351 XbaI*G homozygote/allele and ER-397 PvuII*T heterozygote and allele in the women with endometriosis or leiomyoma compared with normal controls. Mutant variants for both ERα SNPs were correlated with higher susceptibility of endometriosis or leiomyoma. Wild-type allele or homozygote might contribute to decreased illness risks. Our findings were compatible with some previous reports (Table VI), which suggested...
that ERα XbaI and PvuII genes might be associated with the clinical presentation of estrogen-related disorders. The correlations of ERα PvuII T/C polymorphisms with endometriosis or leiomyoma were compatible with previous reports (Georgiou et al., 1999; Kitawaki et al., 2001).

We also observed that the distributions of ERα XbaI and PvuII between endometriosis and leiomyoma were not significantly different, which was compatible with the report of Kitawaki et al. (2001). The similar distributions of ERα genotypes between these two estrogen-dependant disorders suggested their comparable underlying pathogeneses or molecular pathway. However, the distributions of ERα XbaI and PvuII allelic variations in our study were not completely compatible with other studies (Kim et al., 2005). These discrepancies might be due to different illness staging, severities, as well as racial or disease variations. In our study, we recruited individuals with severe endometriosis (AFS stage IV) instead of AFS stage I/II in Kim et al. (2005), which might have resulted in different distributions and conclusions.

There are controversial and inconsistent reports about the ERα-351 XbaI and -397 PvuII polymorphisms with endometriosis and leiomyoma. They might be directly or indirectly correlated with the contributions to the pathogeneses of these gynecological diseases. These findings provide a database for the further survey of the ERα polymorphisms in Asian individuals. Although the real role and mechanism of ERα gene polymorphisms upon these disorders have not yet been clarified, these polymorphisms deserve more attention to realize its importance to endometriosis/leiomyoma development.

References


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