Angiotensin I-Converting Enzyme Insertion-Related Genotypes and Allele are Associated With Higher Susceptibility of Endometriosis and Leiomyoma

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ABSTRACT Endometriosis and leiomyoma display features similar to malignancy, requiring neovascularization to proliferation and growth. Altered vascular-related genes might be related to the development of endometriosis and leiomyoma. Polymorphisms of the angiotensin-converting enzyme (ACE) insertion/deletion (I/D) genes have been linked with some vascular diseases. This study investigates whether ACE I/D gene polymorphisms could be used as markers of susceptibility in endometriosis and leiomyoma. Women were divided into three groups: (1) endometriosis (n = 125); (2) leiomyoma (n = 120); (3) normal controls (n = 128). Genomic DNA was obtained from peripheral leukocyte. ACE I/D gene polymorphisms in intron 16 were amplified by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) Genotypes and allelic frequencies in both groups were compared. We observed the genotype distribution and allele frequency of ACE I/D gene polymorphisms in both groups were significantly different. Proportions of ACE*I homozygote/heterozygote/D homozygote in both groups were: (1) 50.4/24/25.6%; (2) 25/23.33/51.67%; (3) 10.2/29.7/60.1%. Proportions of I/D alleles in each group were: (1) 62.4/37.6%; (2) 36.7/63.3%; (3) 25/75%. We concluded that ACE*I/D gene polymorphisms are associated with endometriosis and leiomyoma susceptibilities. ACE*I-related genotypes and allele are strongly related to the occurrence of endometriosis and moderately related to the occurrence of leiomyoma.

Key Words: angiotensin I-converting enzyme, ACE; endometriosis; leiomyoma; polymorphism

INTRODUCTION

Endometriosis, a polygenic/multifactorial disease, is related with the complex interactions between hormone and cytokines activation, immunoinflammatory process, and genetic factors (Vigano et al., 1998). Endometriosis displays some features of malignancy, including local invasion and aggressive spread to distant organs. Similar to tumor metastases, endometriotic implants require neovascularization to become established, grow, and invade tissues. The neovascular processes are prominent in the endometriosis tissues. Leiomyoma, the most common benign uterine neoplasma, is occurred in around one-fourth of the women during their lifetimes (Cramer, 1992). Leiomyoma is related with the complex mechanism of auto- and paracrine interaction or the effect of sex-steroid hormone action on cells (El-Badry et al., 1991).

Cardiovascular genes play a role in the regulation and growth of tumor. Altered vascular-related genes might be related with the development of endometriosis or leiomyoma. The renin-angiotensin system (RAS) regulates blood pressure through its effects on vascular tone, renal hemodynamics, and fluid–electrolyte balance (Fornage et al., 1998). Renin converts angiotensinogen to angiotensin I. Angiotensin I-converting enzyme (ACE) cleaves angiotensin I to angiotensin II, which is the key component in RAS (Berge and Berg, 1994). ACE regulates the systemic circulation through angiotensin II formation and kinin metabolism. The ACE (encoded by the gene DCP1) and RAS genes are related with the regulatory pathway in cardiovascular disease (Zhu et al., 2001); while the ACE gene is

ACE I-related genotypes and allele are associated with higher susceptibilities of endometriosis and myoma.

Yao-Yuan Hsieh and Cheng-Chun Lee have equal contributions for this survey.

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implicated as a risk factor for coronary artery disease and myocardial infarction (Zhu et al., 2001).

Angiogenesis and vascular remodeling play critical roles in the growth, invasion, and regression of endometriosis or leiomyoma (Donnez et al., 1998). It has been demonstrated that the presence of angiotensin receptors in endometrial tissue (Braileanu et al., 2002). Angiotensin II in endometrial stromal cells was mediated via angiotensin I receptors (Braileanu et al., 2002). Angiotensin II could increase the intracellular calcium concentration by interaction with angiotensin receptor in endometrial stromal cells (Braileanu et al., 2002). Vasopressin also stimulates phospholipase C activity in endometrial explants (Braileanu et al., 2001). These findings suggested the underlying contributions of ACE upon the development of endometriosis and leiomyoma. Heritable genetic factors may contribute to the initiation and progression of endometriosis (Treloar et al., 1999). Gene polymorphisms are useful tools in the study of multifactorial disorders (Anderson et al., 1994). Molecular geneticists are developing the third-generation human genome map with single-nucleotide polymorphisms (SNPs). The analyses of SNPs can be implemented to analyze the mechanisms of complex genetic disorders. Numerous chronic disorders, such as endometriosis, leiomyoma, osteoporosis, hypertension, diabetes, and asthma, have been attributed to genetic susceptibility.

Most studies on ACE gene polymorphisms were focused on their associations with cardiovascular diseases, serum ACE level, and blood pressure (Zhu et al., 2001). Few investigators demonstrated their roles in gynecological diseases, such as endometriosis and leiomyoma. Recently, a 287-bp Alu insertion/deletion in intron 16 of the human gene (Donnez et al., 1998) has been demonstrated that the presence of angiotensin receptors in endometrial tissue (Braileanu et al., 2002). Angiotensin II could increase the intracellular calcium concentration by interaction with angiotensin receptor in endometrial stromal cells (Braileanu et al., 2002). Vasopressin also stimulates phospholipase C activity in endometrial explants (Braileanu et al., 2001). These findings suggested the underlying contributions of ACE upon the development of endometriosis and leiomyoma.

Heritable genetic factors may contribute to the initiation and progression of endometriosis (Treloar et al., 1999). Gene polymorphisms are useful tools in the study of multifactorial disorders (Anderson et al., 1994). Molecular geneticists are developing the third-generation human genome map with single-nucleotide polymorphisms (SNPs). The analyses of SNPs can be implemented to analyze the mechanisms of complex genetic disorders. Numerous chronic disorders, such as endometriosis, leiomyoma, osteoporosis, hypertension, diabetes, and asthma, have been attributed to genetic susceptibility.

Most studies on ACE gene polymorphisms were focused on their associations with cardiovascular diseases, serum ACE level, and blood pressure (Zhu et al., 2001). Few investigators demonstrated their roles in gynecological diseases, such as endometriosis and leiomyoma. Recently, a 287-bp Alu insertion/deletion (I/D) restriction fragment length polymorphism (RFLP) in intron 16 of the human ACE gene has been largely surveyed in individual diseases. However, no investigator demonstrates their association with endometriosis or leiomyoma. Herein, we tried to evaluate the associations of ACE I/D polymorphisms with endometriosis and leiomyoma. To the best of our knowledge, this is the first survey in this field.

MATERIALS AND METHODS

Premenopausal Taiwanese women with surgically diagnosed severe endometriosis, leiomyoma, and normal individuals without endometriosis and leiomyoma were included. The nonendometriosis or nonleiomyoma statuses were confirmed after detail ultrasonography examination. All patients were divided into three groups: (1) severe endometriosis (n = 125, according to revised American Fertility Society classification); (2) leiomyoma (n = 120); (3) normal controls (n = 128). The studies were 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 ACE I/D polymorphism (intron 17) were determined according to previous described condition (Abboud et al., 1998). Genomic DNA was isolated from peripheral blood using Genomaker DNA extractor kit (Blossom, Taipei, Taiwan). About 50 ng of genomic DNA was mixed with 20 pmol of polymerase chain reaction (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 AmpliTag DNA polymerase (Perkin Elmer Applied Biosystems, Foster City, CA). A PCR condition (Taq) was set as follows: one cycle at 94°C for 5 min, 35 cycles at 94°C for 30 sec, 58°C for 45 sec, and final cycle of extension at 72°C for 10 min. After complete PCR processes, the products included a 490-bp fragment with 300-bp insertion for “I” (Insertion) allele and a 190-bp fragment for “D” (Deletion) allele. Each sample revealed one of three electrophoresis patterns: a 490-bp band corresponding to I/I genotype, a 190-bp band corresponding to D/D genotype, or a combination of 490- and 190-bp bands corresponding to I/D genotype.

PCR products (5 µl) were loaded into 3% agarose gel containing ethidium bromide for electrophoresis. Genotypes and allelic frequencies for ACE I/D gene polymorphisms in both groups were compared. Correlations between the ACE I/D genotype and endometriosis/leiomyoma was 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) with χ2 test were utilized for statistical analyses. A P-value of <0.05 was considered statistically significant.

RESULTS

Genotype distribution and allele frequency of ACE I/D gene polymorphisms in three groups were significantly different (Table 1). Higher percentages of I-related genotype (I homozygote, I/D heterozygote) presented in the endometriosis/leiomyoma population compared to normal controls. Proportions of insertion homozygote/heterozygote/deletion homozygote for ACE in three groups were: (1) 125/11/24 (Table 1). Allele frequencies for ACE I/D polymorphism between tumor groups and normal controls were also significantly different (Table 2). Proportions of I/D alleles in both groups were: (1) 62.4/37.6%; (2) 25.3/74.7%; (3) 20.4/79.6%, respectively (P-value < 0.0001, Table 1). Allele frequencies for ACE I/D polymorphism between tumor groups and normal controls were also significantly different (Table 2). Proportions of I/D alleles in both groups were: (1) 62.4/37.6%; (2) 25.3/74.7%; (3) 20.4/79.6%, respectively (P-value < 0.0001, Table 2).

Most common genotype and allele for ACE I/D gene polymorphisms in endometriosis groups were I-related genotype and allele. The I and D distributions were comparable in leiomyoma group. In contrast, the
D-related genotypes were strikingly higher in normal populations (Tables 1 and 2). These findings indicated that ACE*I-related genotype and allele were strongly associated with higher susceptibility of endometriosis as well as moderately correlated with the susceptibility of leiomyoma.

### DISCUSSION

Endometriosis is a common gynecological tumor, but its etiology remains unclear. The prevalence of endometriosis is around 10% in general population (Goldman and Cramer, 1989) and as high as 30–40% in infertile women (Strathy et al., 1982). Numerous hormones and cytokines are associated with endometriosis formation, including estrogen, progesterone (Irahara et al., 2001), insulin-like growth factor (Boehm et al., 1990), epidermal growth factor, fibroblast growth factor (Di Lieto et al., 1997), etc. The endometrium, which has prominent vessels and blood flow, is one of the few adult tissues that exhibit regular intervals of rapid growth and abruption. Therefore, angiogenesis is an important component of the growth and function of these tissues. Endometriosis is a disease of endometrium tissues shedding outside the uterus during menstruation. These explants require a rich blood supply, which enables their survivals and growths. Therefore, the activation of angiogenesis might be a key factor in the pathogenesis of endometriosis (Inan et al., 2003).

Leiomyoma is the most common tumor in women, but the related etiopathogenesis is also obscure. Angiogenesis and vascular-related factors are involved in the pathogenesis and growth of leiomyomas. Uterine tissue contains numerous growth factors, which modified vascular expression and blood supply (Di Lieto et al., 2005). Growth factor could preferentially promote the angiogenesis of leiomyoma cells compared with myometrial cells (Strawn et al., 1995). Leiomyoma contains abnormal vascualization than myometrium does, which may be related to the genesis and progression of these tumors (Boehm et al., 1990). The developments of both endometriosis and leiomyoma are associated with exposure to ovarian sex steroids and increased require of vascular supply for their growth. These observations suggest that angiogenic factors may be related with the development of endometriosis/leiomyoma. However, the vascular-related genes contributing to endometriosis and leiomyoma remain largely unknown.

Endometriosis and leiomyoma development and progression were proposed to be multigenic models. The ACE gene might be linked to other important genes in the process of progression. However, the pathophysiological role of ACE upon endometriosis or leiomyoma is not fully understood. The implications of ACE polymorphisms on endometriosis or leiomyoma remain to be established. It has been suggested that the RAS is involved in the regulation of cell proliferation in the endometrium (Li et al., 2000; Dinh et al., 2001; Schauser et al., 2001; Baudin, 2002). Some steroid hormones might influence the regulation of RAS (Bachman et al., 1991; Harrap et al., 1993). Estrogens increase the hepatic synthesis of renin, which influence the conversion of angiotensinogen to angiotensin I as well as the following catalyses of ACE (Harrap et al., 1993). Morever, one of angiotensin II receptors (subtype 2) is abundant in the uterus tissue (Dinh et al., 2001; Baudin, 2002). Therefore, it is logical to suspect that RAS might be involved in endometriosis or leiomyoma.

ACE catalyses the conversion of angiotensin I to the physiologically active peptide angiotensin II, which controls fluid–electrolyte balance and systemic blood

### TABLE 1. Distributions of ACE I/D Gene Polymorphisms in Women With Endometriosis, Leiomyoma, and Normal Controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Endometriosis (n = 125) (%)</th>
<th>Leiomyoma (n = 120) (%)</th>
<th>Normal controls (n = 128) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insertion/insertion</td>
<td>63 (50.4)</td>
<td>30 (25)</td>
<td>13 (10.2)</td>
</tr>
<tr>
<td>Insertion/deletion</td>
<td>30 (24)</td>
<td>28 (23.33)</td>
<td>38 (29.7)</td>
</tr>
<tr>
<td>Deletion/deletion</td>
<td>32 (25.6)</td>
<td>62 (51.67)</td>
<td>77 (60.1)</td>
</tr>
</tbody>
</table>

*P-value was calculated by χ² test.
*P-value < 0.000001.
**P-value = 0.008.
***P-value = 0.000024.

### TABLE 2. Allelic Frequencies for ACE I/D Polymorphisms in Women With Endometriosis, Leiomyoma, and Normal Controls

<table>
<thead>
<tr>
<th>Allele frequencies</th>
<th>Endometriosis (n = 250) (%)</th>
<th>Leiomyoma (n = 240) (%)</th>
<th>Normal controls (n = 256) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insertion</td>
<td>156 (62.4)</td>
<td>88 (36.7)</td>
<td>64 (25)</td>
</tr>
<tr>
<td>Deletion</td>
<td>94 (37.6)</td>
<td>152 (63.3)</td>
<td>192 (75)</td>
</tr>
</tbody>
</table>

*P-values were calculated by χ² test.
*P-value < 0.00001.
**P-value = 0.0048.
***P-value = 0.000001.
pressure. ACE activity is related with angiogenesis. ACE inhibition by perindopril improves myocardial angiogenesis (Toblli et al., 2004). Angiotensin II, a key regulator of blood pressure and body fluid homeostasis, exerts mitogenic effects on endothelial cells. Angiotensin II has been shown to have possible mitogenic and angiogenic effects upon tumor cell lines (Koh et al., 2003). Angiotensin II is also a humoral regulator of peripheral angiogenesis (Walther et al., 2003). Because ACE catalyzed the angiotensin II formation, the ACE activity is positively correlated with angiotensin II production. Furthermore, ACE inhibitors were found to significantly inhibit tumor growth and angiogenesis along with suppression of the vascular endothelial growth factor (VEGF) level (Yasumatsu et al., 2004).

Recently, it has been reported that ACE polymorphisms may be determinants in the development of human cancers (Haiman et al., 2003; Koh et al., 2003; Medeiros et al., 2004). The long-term use of ACE may protect against cancer (Lever et al., 1998). The ACE inhibitor (e.g., captopril) has been shown to inhibit proliferation of tumor cells and to reduce tumor growth (Reddy et al., 1995; Volpert et al., 1996; Small et al., 1997; Hii et al., 1998). Angiotensin II might have an important role in carcinogenesis, and the antiangiogenic activity is partly mediated by angiotensin II and ACE inhibition. ACE and angiotensin II inhibitors might be considered as useful anti-tumor agents. Combined these presentations, it is logical to expect that ACE might appear to be an angiogenic and tumorogenesis effects upon endometriosis and leiomyoma.

The ACE gene, which is located on chromosome 17q23, contains some gene polymorphisms and candidate markers for hypertension and related diseases (Doria et al., 1994). The ACE gene polymorphism located on the intron 17 of ACE gene. ACE gene mediates interaction effects of the fibrinolytic and RASs on plasma levels of plasminogen activator inhibitor 1 (PAI-1) (Moore et al., 2002). It might contain linkage disequilibrium with other important gene variant. ACE gene polymorphisms are related to numerous diseases, including coronary heart disease, hypertension, ventricular hypertrophy (Alvarez et al., 2000; Henskens et al., 2003; Sethi et al., 2003), systemic lupus erythematosus, nephritis (Parsa et al., 2002), carotid artery wall thickness (Sayed-Tabatabaei et al., 2003), post-transplant erythrocytosis (Yildiz et al., 2003), diabetic nephropathy (Chang et al., 2003), Alzheimer’s disease (Kehoe et al., 2003), ischemic cerebrovascular disease (Um et al., 2003), dementia (Choi et al., 2003), segmental glomerulosclerosis (Dixit et al., 2002), cystic fibrosis (Arkwright et al., 2003), sarcoidosis (Schurmann et al., 2001) etc.

Most studies have associated the presence (insertion, I) or absence (deletion, D) of a 287-bp Alu repeat element in intron 16 with the levels of circulating enzyme or cardiovascular pathophysiology. ACE I/D polymorphism affects the uteroplacental and umbilical flows as well as the recurrence of an adverse pregnancy outcome in women with pre-eclampsia (Mello et al., 2003). Individuals with the DD genotype show a significantly increased left-ventricular mass in response to physical training, compared to the II or ID genotype as well as the lowest plasma ACE levels (Alvarez et al., 2000). ACE I/D genotype DD might be a promoter to clinical manifestation of sarcoidosis. A significant association was observed between the presence of ACE*D genotype/ allele and an elevation of serum ACE activity (Martinez et al., 2000).

Despite many epidemiological studies suggest that the ACE*D allele confers increased susceptibility to cardiovascular disease; however, other reports have found no such association or even a beneficial effect (Rieder et al., 1999). ACE polymorphism may be associated with the development of endometrial carcinoma (Freitas-Silva et al., 2004). The presence of I allele (genotypes ID and II) is significantly associated to an earlier age of onset of endometrial carcinoma (Freitas-Silva et al., 2004). In contrast, the D-related genotype (DD, ID) exhibited higher risk of breast cancer (Koh et al., 2003) and advanced progression of prostate cancer (Medeiros et al., 2004). Furthermore, some investigators have demonstrated the nonassociations between the ACE gene polymorphisms with individual diseases, including hypertension (Harrap et al., 1993; Berge and Berg, 1994; Mondorf et al., 1998), myocardial infarction (Foy et al., 1997), left ventricular hypertrophy (West et al., 1997), pregnancy outcomes, pregnancy-induced hypertension (Tamura et al., 1996), nephronophthisis (Omran et al., 1999), and sarcoidosis (Schurmann et al., 2001).

There is controversial and inconsistent reports about the ACE I/D distributions in individual diseases among different races (Table 3). The D allele has a frequency of approximately 0.53 in Caucasians (Cambien, 1994) in

| TABLE 3. Distributions of ACE Insertion/Deletion (I/D) Alleles for Individual Diseases |
|---------------------------------|---------------------------------|---------------------------------|
| Literatures                     | I/D (%) for individual diseases | I/D (%) for normal controls    |
| Zee et al. (1992)               | 56/44 (hypertension)            | 41/59                           |
| Yamamoto et al. (1997)          |                                | 64/36 (children)                |
| Isbir et al. (1999)             | 62.3/37.7 (coronary artery disease) | 49.3/50.7                     |
| Nakai et al. (1994)             | 42/58 (coronary artery disease) | 58/42                           |
| Moriyama et al. (1995)          | 81/19 (non-effective patients with proteinuria after ACE inhibitors therapy) | 53/47 (effective patients with proteinuria after ACE inhibitors therapy) |
| Matsubara et al. (2002)         | 67.5/22.5 (hypertension)        |                                 |

Significant difference.
Nonsignificant difference between systolic/diastolic pressure.
contrasted to a 0.42 in Asians (Hiramori, 1994). These discrepancies might be due to different illness classifications, racial or disease variations. In fact, different ethnic groups might influence the ACE gene distributions (McKenzie et al., 2001). Ethnic variation plays a major role in genetic regulation of serum ACE activity and ACE gene polymorphism to cardiovascular disease (Bloem et al., 1996). Furthermore, the gender-specific influence of these gene polymorphisms should be concerned in the relative surveys.

In our previous survey, we demonstrated the correlations of ACE A2350G and A-2240T gene polymorphisms in endometriosis (Hsieh et al., 2005). We observed that ACE 2350G and ACE-240T-related genotypes and alleles are associated with higher susceptibilities of endometriosis. In this study, we further observed that the genotype distributions and allele frequencies for ACE I/D polymorphisms were significantly different between the individuals with and without endometriosis/leiomyoma. The ACE*I-related genotype and allele are strongly related to the occurrence of endometriosis, compared to being moderately correlated with the occurrence of leiomyoma. Our results firstly indicated the ACE gene polymorphisms might predispose to endometriosis or leiomyoma developments. This finding is also compatible with some previous reports (Table 3), who suggested that the ACE*I allele might be associated with higher susceptibility of individual diseases. Therefore, our data strongly suggests that the ACE gene polymorphisms might substantially contribute to the pathogenesis of endometriosis or leiomyoma. It also suggests that RAS might be involved in the pathogenesis of these diseases.

The mechanisms of SNPs upon individual disease remain uncertain. Despite the SNPs do not alter the transcript levels, some investigator demonstrated the disequilibrium effects of certain genotypes might influence the related 3-dimensional structure and efficiency of the transcripts (Shintani et al., 1999; Kennon et al., 2004; Shirasawa et al., 2004). Presumably, the distinct biological condition caused by ACE is among the numerous contributions, which influence the endometriosis developments. These contributions include genetic, dietary, and environmental regulating hormonal and nonhormonal conditions. Furthermore, the ACE polymorphisms might be in linkage disequilibrium with other unidentified functional polymorphisms, which cooperatively influences the endometriosis susceptibility.

In conclusion, associations of ACE*I/D gene polymorphisms with endometriosis and leiomyoma exist. ACE*I related genotype and alleles increase the susceptibility to endometriosis as well as leiomyoma. The ACE gene polymorphisms likely contribute to the pathogenesis of these gynecological diseases. Although the real role and mechanism of ACE gene polymorphism upon these disorders has not yet been clarified, this polymorphism deserves more attention to realize its importance to endometriosis/leiomyoma development. Furthermore, this study could be extended to determine whether the RAS and its related gene polymorphisms also affect the endometriosis or leiomyoma formation. After the clarification of its role upon endometriosis and leiomyoma, ACE gene polymorphism may become a useful marker to predict the future development of these diseases and to permit early therapeutic intervention in women at high risk for endometriosis or leiomyoma.

REFERENCES


