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# Estrogen receptor α-351 *Xba*I*G and -397 *Pvu*II*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 (*Xba*I) and -397 T>C (*Pvu*II) 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 *Xba*I and -397 T/C *Pvu*II 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) 33.6/64.6/1.8 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 *Xba*I*G- and -397 *Pvu*II*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 neoplasma 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

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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 analyse the mechanisms of complex genetic disorders.

ER is also involved in metabolic pathways influencing estrogen-related tissue growth and height stature (Schuit *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. Allelic 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 *Xba*I (dbSNP: rs9340799) and *Pvu*II (dbSNP: rs2234693) (Ioannidis *et al*., 2004; van Duijnhoven *et al*., 2005). The associations between the ERα polymorphism and breast carcinoma 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 *Xba*I and *Pvu*II 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 *Pvu*II and *Xba*I polymorphism in Taiwanese women with endometriosis or leiomyoma.

## Materials and methods

Premenopausal Taiwanese women with surgically diagnosed severe endometriosis [Revised American Fertility Society (AFS) 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 examination. The non-endometriosis or non-leiomyoma statuses were 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 *Xba*I A/G (uncuttable/cuttable) and -397 *Pvu*II T/C (uncuttable/cuttable) 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 $MgCl_2$, 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 (*Xba*I and *Pvu*I; 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 *Xba*I and *Pvu*II polymorphisms were termed AA/AG/GG and TT/TC/CC, respectively. Genotypes and allelic frequencies for ERα *Xba*I A/G and *Pvu*II T/C gene polymorphisms in each group were compared. Correlations of ERα *Xba*I A/G and *Pvu*II 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 $\chi^2$ test was utilized for statistical analyses. A $P$-value <0.05 was considered statistically significant.

## Results

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

**Table I.** The primer sequences, PCR and restriction fragment length polymorphism (RFLP) conditions for estrogen receptor α (ERα)-351 A/G *Xba*I and -397 T/C *Pvu*II gene polymorphisms

| Polymorphisms (locations) | Primers sequences (5′→3′) | Denature (°C/s) | Annealing (°C/s) | Extension (°C/s) | Restriction enzyme (°C/min) | SNP sequence | Allelic variants | DNA fragment size (bp) |
|---|---|---|---|---|---|---|---|---|
| ERα-351 A/G XbaI polymorphism | F-CTGCCACCCTATCTGTATCTTTTCCTATTCTCC | 94/30 | 58/30 | 72/30 | XbaI (37/60) | A (wild) | A | 1374 |
| | | | | | | G (mutant) | G | 982 + 392 |
| ERα-397 T/C PvuII polymorphism | R-TCTTTCTCTGCCACCCTGGCGTCGATTATCTGA | 94/30 | 58/30 | 72/30 | PvuII (65/60) | T (wild) | T | 1374 |
| | | | | | | C (mutant) | C | 937 + 437 |

SNP, single-nucleotide polymorphism.

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

| Genotypes | Endometriosis ($n$ = 112[a,c]) | Leiomyoma ($n$ = 106[a,b]) | Normal controls ($n$ = 110[b,c]) |
|---|---|---|---|
| AA homozygote | 30 (26.8%) | 21 (19.8%) | 37 (33.6%) |
| AG heterozygote | 64 (57.1%) | 56 (52.8%) | 71 (64.6%) |
| GG homozygote | 18 (16.1%) | 29 (27.4%) | 2 (1.8%) |

[a]Not statistically different (endometriosis versus leiomyoma).
[b]$P < 0.00005$ (leiomyoma versus controls).
[c]$P < 0.005$ (endometriosis versus controls).

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

| Allelic variants | Endometriosis ($n$ = 224[a,c]) | Leiomyoma ($n$ = 212[a,b]) | Normal controls ($n$ = 220[b,c]) |
|---|---|---|---|
| A allele | 124 (55.4%) | 98 (46.2%) | 145 (65.9%) |
| G allele | 100 (44.6%) | 114 (53.8%) | 75 (34.1%) |

A allele, wild uncuttable type; G allele, mutant cuttable type.
[a]Not statistically different (endometriosis versus leiomyoma).
[b]$P < 0.00005$ (leiomyoma versus controls).
[c]$P < 0.005$ (endometriosis versus controls).

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

| Genotypes | Endometriosis ($n$ = 112[a,c]) | Leiomyoma ($n$ = 106[a,b]) | Normal controls ($n$ = 110[b,c]) |
|---|---|---|---|
| TT homozygote | 27 (24.1%) | 25 (23.6%) | 60 (54.5%) |
| TC heterozygote | 68 (60.7%) | 75 (70.8%) | 44 (40.0%) |
| CC homozygote | 17 (15.2%) | 6 (5.6%) | 6 (5.5%) |

[a]Not statistically different (endometriosis versus leiomyoma).
[b]$P < 0.00005$ (leiomyoma versus controls).
[c]$P < 0.00005$ (endometriosis versus controls).

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

| Allelic variants | Endometriosis ($n$ = 224[a,c]) | Leiomyoma ($n$ = 212[a,b]) | Normal controls ($n$ = 220[b,c]) |
|---|---|---|---|
| T allele | 122 (54.5%) | 125 (59.0%) | 164 (74.5%) |
| C allele | 102 (45.5%) | 87 (41.0%) | 56 (25.5%) |

C allele, mutant cuttable type; T allele, wild uncuttable type.
[a]Not statistically different (endometriosis versus leiomyoma).
[b]$P < 0.005$ (leiomyoma versus controls).
[c]$P < 0.00005$ (endometriosis versus controls).

with a higher susceptibility of endometriosis/leiomyoma. Proportions of ERα *Xba*I*AA/AG/GG in each group were (i) 26.8/57.1/16.1%, (ii) 19.8/52.8/27.4% and (iii) 33.6/64.6/1.8%, respectively (Tables II and III). Proportions of ERα *Xba*I*A/G alleles in each group were (i) 55.4/44.6%, (ii) 46.2/53.8% and (iii) 65.9/34.1%, respectively.

The most common genotypes and allele for *Pvu*II gene polymorphisms in each group were T-related genotypes and allele. ERα *Pvu*II*C-related genotype and allele were associated with higher susceptibility of endometriosis/leiomyoma. Proportions of ERα *Pvu*II TT/TC/CC in each groups were (i) 24.1/60.7/15.2%, (ii) 23.6/70.8/5.6% and (iii) 54.5/40.0/5.5%, respectively (Tables IV and V). Proportions of ERα *Pvu*II T/C alleles in each group were (i) 54.5/45.5%, (ii) 59.0/41.0% and (iii) 74.5/25.5%, respectively. These findings indicate that ERα *Pvu*II and *Xba*I mutant genotype and allele are strongly associated with higher susceptibility of endometriosis or 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 (*Pvu*II and *Xba*I) are located in the first intron of ERα gene (Kobayashi *et al*., 1996). ERα *Xba*I and *Pvu*II gene polymorphisms have been reported to be related to numerous estrogen-related diseases (Table VI). Erα-mutant alleles (*Xba*I*G and *Pvu*II*C) were associated with elevated serum estradiol ($E_2$) production (Zofkova *et al*., 2002; Schuit *et al*., 2005). Such relationships provide the molecular pathways between ERα allelic variations and endometriosis/leiomyoma pathogeneses.

In this study, we observed that the genotype distributions and allele frequencies for ERα *Xba*I A/G and *Pvu*II 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 *Xba*I and *Pvu*II gene polymorphisms might predispose to endometriosis or leiomyoma development. The ERα *Xba*I -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 *Pvu*II -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 *Xba*I*G homozygote/allele and ER-397 *Pvu*II*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

**Table VI.** Correlations of estrogen receptor α (ERα)-351 *Xba*I and -397 *Pvu*II gene polymorphisms with individual diseases

| SNP | Correlation | Non-correlation |
|---|---|---|
| | Diseases and references | Diseases and references |
| –351 *Xba*I and -397 *Pvu*II | Age of menarche (Stavrou *et al*., 2006)<br>Symptom of menopause (Malacara *et al*., 2004)<br>Breast cancer (Boyapati *et al*., 2005; van Duijnhoven *et al*., 2005)<br>Osteoporosis (Lorentzon *et al*., 1999; Massart, 2005)<br>Height stature (Schuit *et al*., 2004a)<br>Osteoarthritis (Jin *et al*., 2004)<br>Prostate cancer (Hernandez *et al*., 2006)<br>Trigeminal neuralgia (Huang *et al*., 2005)<br>Adiposity (Huang *et al*., 2005)<br>Systemic lupus erythematosus (Johansson *et al*., 2005)<br>Cholesterol metabolism (Kajinami *et al*., 2005)<br>Alzheimer's disease (den Heijer *et al*., 2004)<br>Ischemic heart disease (Schuit *et al*., 2004b)<br>Paget's disease (Donath *et al*., 2004)<br>Aortic valve sclerosis (Nordstrom *et al*., 2003) | Endometriosis (Wang *et al*., 2004; Kim *et al*., 2005)<br>Menarche (Xu *et al*., 2005)<br>Osteoporosis (Dennison *et al*., 2005; Jian *et al*., 2005) |
| –351 *Xba*I | Clinical course of thalassemia (Iarussi *et al*., 2005)<br>Breast cancer (Lu *et al*., 2005) | – |
| –397 *Pvu*II | Endometriosis (Georgiou *et al*., 1999; Kitawaki *et al*., 2001)<br>Leiomyoma (Kitawaki *et al*., 2001)<br>Adenomyosis (Kitawaki *et al*., 2001)<br>Breast cancer (Onland-Moret *et al*., 2005)<br>Neurofibrillary tangles (Kazama *et al*., 2004) | Breast cancer (Lu *et al*., 2005) |

SNP, single-nucleotide polymorphism.

that ERα *Xba*I and *Pvu*II genes might be associated with the clinical presentation of estrogen-related disorders. The correlations of ERα *Pvu*II 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 *Xba*I and *Pvu*II 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 *Xba*I and *Pvu*II allelic variations in our study were not completely compatible with other studies (Kim *et al*., 2005). These discrepancies might be due to different illness stagging, 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 *Xba*I*G and -397 *Pvu*II*C-related genotype distributions or association in individual diseases among different races (Table VI). Ethnic variation plays a major role in genetic regulation of estrogen or ER activity and related polymorphism to individual diseases. Furthermore, the gender-specific influence of these gene polymorphisms should be considered in the relative studies. In the study of Lorentzon *et al*. (1999), the distributions of *Xba*I AA/AG/GG and *Pvu*II TT/TC/CC in 90 Caucasians boys were 8.9/40.0/51.1 and 22.2/44.5/33.3%, respectively. In contrast, in one study with 174 post-menopausal Korean women, Nam *et al*. (2005) demonstrated the related distributions were 3.5/29.3/67.2 and 14.9/46.0/39.1%, respectively. These incompetences might be due to racial, ethical or gender variation as well as illness classification.

The mutant alleles for these two SNPs might be serving as markers of a functional variant in a nearby gene. This study could be extended to understand whether the ERα mutation also affects ERα function and endometriosis formation. After the clarification of their correlations and role upon endometriosis and leiomyoma, ERα gene polymorphism may become a useful marker to predict the future susceptibility of these diseases and to permit early therapeutic intervention in women at high risk for endometriosis or leiomyoma. A further promising application of these polymorphisms comes from their pharmacogenomic implications, with the possibility of providing better guidance for therapeutic regimens, such as selective ER modulators and ER antagonist transfection therapy.

In conclusion, associations of ERα-351 *Xba*I and -397 *Pvu*II polymorphisms with endometriosis and leiomyoma exist. ERα *Xba*I*G- and *Pvu*II*C-related genotypes/alleles are correlated with higher susceptibilities of endometriosis or leiomyoma, which provided a useful tool in predicting endometriosis/leiomyoma susceptibility. ERα gene polymorphisms might influence the development of endometriosis or leiomyoma in Taiwanese women. They might be directly or indirectly correlated with the contributions to the pathogeneses of these gynaecological 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

Andersen J and Barbieri RL (1995) Abnormal gene expression in uterine leiomyomas. J Soc Gynecol Invest 2,663–672.

Anderson TI, Heimdal KR, Skrede M, Tveit K, Berg K and Borresen AL (1994) Oestrogen receptor (ESR) polymorphisms and breast cancer susceptibility. Hum Genet 94,665–670.

Boyapati SM, Shu XO, Ruan ZX, Cai Q, Smith JR, Wen W, Gao YT and Zheng W (2005) Polymorphisms in ER-alpha gene interact with estrogen receptor status in breast cancer survival. Clin Cancer Res 11,1093–1098.

Carstens RP, McKeehan WL and Garcia-Blanco MA (1998) An intronic sequence element mediates both activation and repression of rat fibroblast growth factor receptor 2 pre-mRNA splicing. Mol Cell Biol 18,2205–2217.

Cramer DW (1992) Epidemiology of myomas. Semin Reprod Endocrinol 10,320–324.

Dennison E, Syddall H, Fall C, Brandi ML, Cooper C; Hertfordshire Cohort Study Group (2005) Evidence of sexual dimorphism in relationships between estrogen receptor polymorphisms and bone mass: the Hertfordshire study. J Rheumatol 32,2400–2404.

Donath J, Speer G, Poor G, Gergely P Jr, Tabak A and Lakatos P (2004) Vitamin D receptor, oestrogen receptor-alpha and calcium-sensing receptor genotypes, bone mineral density and biochemical markers in Paget's disease of bone. Rheumatology (Oxford) 43,692–695.

van Duijnhoven FJ, Bezemer ID, Peeters PH, Roest M, Uitterlinden AG, Grobbee DE and van Gils CH (2005) Polymorphisms in the estrogen receptor alpha gene and mammographic density. Cancer Epidemiol Biomarkers Prev 14,2655–2660.

Dunning AM, Healey CS, Pharoah PD, Teare MD, Ponder BA and Easton DF (1999) A systematic review of genetic polymorphisms and breast cancer risk. Cancer Epidemiol Biomarkers Prev 8,843–854.

El-Badry OM, Helman LJ, Chatten J, Steinberg SM, Evans AE and Israel MA (1991) Insulin-like growth factor II-mediated proliferation of human neuroblastoma. J Clin Invest 87,648–657.

Gasch A, Hinz U and Renkawitz-Pohl R (1989) Intron and upstream sequences regulate expression of the Drosophila b3-tubulin gene in the visceral and somatic musculature, respectively. Proc Natl Acad Sci USA 86,3215–3218.

Georgiou I, Syrrou M, Bouba I, Dalkalitsis N, Paschopoulos M, Navrozoglou I and Lolis D (1999) Association of estrogen receptor gene polymorphisms with endometriosis. Fertil Steril 72,164–166.

Goldman MB and Cramer DW (1989) Current concepts in endometriosis. Prog Clin Biol Res 323,17–23.

den Heijer T, Schuit SC, Pols HA, van Meurs JB, Hofman A, Koudstaal PJ, van Duijn CM, Uitterlinden AG and Breteler MM (2004) Variations in estrogen receptor alpha gene and risk of dementia, and brain volumes on MRI. Mol Psychiatry 9,1129–1135.

Hernandez J, Balic I, Johnson-Pais TL, Higgins BA, Torkko KC, Thompson IM and Leach RJ (2006) Association between an estrogen receptor alpha gene polymorphism and the risk of prostate cancer in black men. J Urol 175,523–527.

Hsieh YY, Chang CC, Tsai FJ, Wu JY, Tsai CC and Tsai HD (2001) Androgen receptor trinucleotide polymorphism in endometriosis. Fertil Steril 76,412–413.

Hsieh YY, Chang CC, Tsai FJ, Tsai HD, Yeh LS, Lin CC and Tsai CH (2003) Estrogen receptor thymine-adenine dinucleotide repeat polymorphism is associated with susceptibility to leiomyoma. Fertil Steril 79,96–99.

Hsieh YY, Chang CC, Tsai FJ, Lin CC, Yeh LS and Peng CT (2004) Androgen receptor trinucleotide polymorphism in leiomyoma. J Assist Reprod Genet 21,453–457.

Hsieh YY, Chang CC, Tsai FJ, Lin CC and Tsai CH (2005a) Estrogen receptor alpha dinucleotide repeat and cytochrome P450c17alpha gene polymorphisms are associated with susceptibility to endometriosis. Fertil Steril 83,567–572.

Hsieh YY, Chen IP, Wang HI, Chang CC, Huang CW and Lin CS (2005b) PROGINS Alu sequence insertion is associated with hyperprolactinemia but not leiomyoma susceptibility. Clin Endocrinol 62,492–497.

Huang CJ, Wang H, Wu MY, Zhang JJ, Meng QR, Fu CH, Deng JZ and Yi P (2005) Association of estrogen receptor gene polymorphisms and primary trigeminal neuralgia. Hua Xi Kou Qiang Yi Xue Za Zhi 23,495–497.

Iarussi D, Capozzi L and Esposito L (2005) Impact of excess weight and estrogen receptor gene polymorphisms on clinical course of homozygous beta thalassemia. Hematology 10,407–411.

Ioannidis JP, Ralston SH, Bennett ST, Brandi ML, Grinberg D, Karassa FB, Langdahl B, van Meurs JB, Mosekilde L, Scollen S *et al*. (2004) Differential genetic effects of ESR1 gene polymorphisms on osteoporosis outcomes. JAMA 292,2105–2114.

Irahara M, Uemura H, Yasui T, Kinoshita H, Yamada M, Tezuka M, Kiyokawa M, Kamada M and Aono T (2001) Efficacy of every-other-day administration of conjugated equine estrogen and medroxyprogesterone acetate on gonadotropin-releasing hormone agonists treatment in women with endometriosis. Gynecol Obstet Invest 52,217–222.

Jian WX, Yang YJ, Long JR, Li YN, Deng FY, Jiang DK and Deng HW (2005) Estrogen receptor alpha gene relationship with peak bone mass and body mass index in Chinese nuclear families. J Hum Genet 50,477–482.

Jin SY, Hong SJ, Yang HI, Park SD, Yoo MC, Lee HJ, Hong MS, Park HJ, Yoon SH, Kim BS *et al*. (2004) Estrogen receptor-alpha gene haplotype is associated with primary knee osteoarthritis in Korean population. Arthritis Res Ther 6,R415–R421.

Johansson M, Arlestig L, Moller B, Smedby T and Rantapaa-Dahlqvist S (2005) Oestrogen receptor {alpha} gene polymorphisms in systemic lupus erythematosus. Ann Rheum Dis 64,1611–1617.

Kajinami K, Brousseau ME, Lamon-Fava S, Ordovas JM and Schaefer EJ (2005) Gender-specific effects of estrogen receptor alpha gene haplotype on high-density lipoprotein cholesterol response to atorvastatin: interaction with apolipoprotein AI gene polymorphism. Atherosclerosis 178,331–338.

Kazama H, Ruberu NN, Murayama S, Saito Y, Nakahara K, Kanemaru K, Nagura H, Arai T, Sawabe M, Yamanouchi H *et al*. (2004) Association of estrogen receptor alpha gene polymorphisms with neurofibrillary tangles. Dement Geriatr Cogn Disord 18,145–150.

Kennedy S (1998) The genetics of endometriosis. J Reprod Med 43,263–268.

Kennon B, Ingram MC, Friel EC, Anderson NH, MacKenzie SM, Davies E, Shakerdi L, Wallace AM, Fraser R and Connell JM (2004) Aldosterone synthase gene variation and adrenocortical response to sodium status, angiotensin II and ACTH in normal male subjects. Clin Endocrinol (Oxf) 61,174–181.

Kim SH, Choi YM, Jun JK, Kim SH, Kim JG and Moon SY (2005) Estrogen receptor dinucleotide repeat polymorphism is associated with minimal or mild endometriosis. Fertil Steril 84,774–777.

Kitawaki J, Obayashi H, Ishihara H, Koshiba H, Kusuki I, Kado N, Tsukamoto K, Hasegawa G, Nakamura N and Honjo H (2001) Oestrogen receptor-alpha gene polymorphism is associated with endometriosis, adenomyosis and leiomyomata. Hum Reprod 16,51–55.

Kobayashi S, Inoue S, Hosoi T, Ouchi Y, Shiraki M and Orimo H (1996) Association of bone mineral density with polymorphism of the estrogen receptor gene. J Bone Miner Res 11,306–311.

Liu J, Zhu H and Zhu X (2001) The effect of estrogen receptor gene Px haplotype on bone mineral density in Chinese postmenopausal women. Zhonghua Yi Xue Za Zhi 81,1295–1297.

Lorentzon M, Lorentzon R, Backstrom T and Nordstrom P (1999) Estrogen receptor gene polymorphism, but not estradiol levels, is related to bone density in healthy adolescent boys: a cross-sectional and longitudinal study. J Clin Endocrinol Metab 84,4597–4601.

Lu X, Li B, Wei JM and Hua B (2005) The XbaI and PvuII gene polymorphisms of the estrogen receptor alpha gene in Chinese women with breast cancer. Zhonghua Wai Ke Za Zhi 43,290–293.

Malacara JM, Perez-Luque EL, Martinez-Garza S and Sanchez-Marin FJ (2004) The relationship of estrogen receptor-alpha polymorphism with symptoms and other characteristics in post-menopausal women. Maturitas 49,163–169.

Massart F (2005) Human races and pharmacogenomics of effective bone treatments. Gynecol Endocrinol 20,36–44.

Nam HS, Shin MH, Kweon SS, Park KS, Sohn SJ, Rhee JA, Choi JS and Son MH (2005) Association of estrogen receptor-alpha gene polymorphisms with bone mineral density in postmenopausal Korean women. J Bone Miner Metab 23,84–89.

Nisolle M, Casanas-Roux F and Donnez J (1997) Immunohistochemical analysis of proliferative activity and steroid receptor expression in peritoneal and ovarian endometriosis. Fertil Steril 68,912–919.

Nordstrom P, Glader CA, Dahlen G, Birgander LS, Lorentzon R, Waldenstrom A and Lorentzon M (2003) Oestrogen receptor alpha gene polymorphism is related to aortic valve sclerosis in postmenopausal women. J Intern Med 254,140–146.

Onland-Moret NC, van Gils CH, Roest M, Grobbee DE and Peeters PH (2005) The estrogen receptor alpha gene and breast cancer risk (The Netherlands). Cancer Causes Control 16,1195–1202.

Revised American Fertility Society classification of endometriosis: 1985 (1985) Fertil Steril 43, 351–352.

Sano, M, Inoue, S, Hosoi, T, Ouchi, Y, Emi, M, Shiraki, M and Orimo, H (1995) Association of estrogen receptor dinucleotide repeat polymorphism with osteoporosis. Biochem Biophys Res Commun 217,378–383.

Schuit SC, van Meurs JB, Bergink AP, van der Klift M, Fang Y, Leusink G, Hofman A, van Leeuwen JP, Uitterlinden AG and Pols HA (2004a) Height in pre- and postmenopausal women is influenced by estrogen receptor alpha gene polymorphisms. J Clin Endocrinol Metab 89,303–309.

Schuit SC, Oei HH, Witteman JC, Geurts van Kessel CH, van Meurs JB, Nijhuis RL, van Leeuwen JP, de Jong FH, Zillikens MC, Hofman A *et al*. (2004b) Estrogen receptor alpha gene polymorphisms and risk of myocardial infarction. JAMA 291,2969–2977.

Schuit SC, de Jong FH, Stolk L, Koek WN, van Meurs JB, Schoofs MW, Zillikens MC, Hofman A, van Leeuwen JP, Pols HA *et al*. (2005) Estrogen receptor alpha gene polymorphisms are associated with estradiol levels in postmenopausal women. Eur J Endocrinol 153,327–334.

Shintani S, Matsuo K, Crohin CC, McBride J, Tsuji T, Donoff RB, Posner M, Todd R and Wong DT (1999) Intragenic mutation analysis of the human

epidermal growth factor receptor (EGFR) gene in malignant human oral keratinocytes. Cancer Res 59,4142–4147.

Shirasawa S, Harada H, Furugaki K, Akamizu T, Ishikawa N, Ito K, Ito K, Tamai H, Kuma K, Kubota S *et al*. (2004) SNPs in the promoter of a B cell-specific antisense transcript, SAS-ZFAT, determine susceptibility to autoimmune thyroid disease. Hum Mol Genet 13,2221–2231.

Stavrou I, Zois C, Chatzikyriakidou A, Georgiou I and Tsatsoulis A (2006) Combined estrogen receptor {alpha} and estrogen receptor beta genotypes influence the age of menarche. Hum Reprod 21,554–557.

Strathy JH, Molgaard CA, Coulam CB and Melton LJ III (1982) Endometriosis and infertility: a laparoscopic study of endometriosis among fertile and infertile women. Fertil Steril 38,667–672.

Treloar SA, O'Connor DT, O'Connor VM and Martin NG (1999) Genetic influences on endometriosis in an Australian twin sample. Fertil Steril 71,701–710.

Urabe M, Yamamoto T, Naito K, Kitawaki J, Honjo H and Okada H (1990) Study on the local estrogen biosynthesis in human uterine leiomyoma. Nippon Sanka Fujinka Gakkai Zasshi 42,1229–1236.

Wang Z, Yoshida S, Negoro K, Kennedy S, Barlow D and Maruo T (2004) Polymorphisms in the estrogen receptor beta gene but not estrogen receptor alpha gene affect the risk of developing endometriosis in a Japanese population. Fertil Steril 81,1650–1656.

Xu H, Long JR, Li MX and Deng HW (2005) Interaction effects between estrogen receptor alpha and vitamin D receptor genes on age at menarche in Chinese women. Acta Pharmacol Sin 26,860–864.

Zofkova I, Zajickova K and Hill M (2002) The estrogen receptor alpha gene determines serum androstenedione levels in postmenopausal women. Steroids 67,815–819.

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