Estrogen receptor gene polymorphisms are associated with recurrence of endometriosis

The presence of gene polymorphisms of the estrogen receptors ERα (*Pvu*II and *Xba*I) and ERβ (*Alu*I) in 61 women with endometriosis was investigated. A statistically significant correlation between *Pvu*II ERα gene polymorphism (PvuII), both in homozygosity (PP) and in heterozygosity (Pp), and a recurrence of endometriosis was found. In conclusion, women affected by endometriosis with the ERα polymorphic allele, even if heterozygous, have a worse prognosis, and these results suggest that the ERα gene polymorphisms may be included among the genetic risk factors for endometriosis. (Fertil Steril® 2006;85:764–6. ©2006 by American Society for Reproductive Medicine.)

Endometriosis is defined as the presence of endometrial glands and stroma outside the uterine cavity, and 5%–10% of women of reproductive age are estimated to be affected by this pathology. Pain, including dysmenorrhea, deep dyspareunia, and chronic pelvic pain, as well as infertility are associated with endometriosis (1).

Because endometriosis is prevalent in women of reproductive age and regresses after menopause or ovariectomy, it is considered an estrogen (E)-dependent disease. In fact, a decrease in serum E levels at menopause or caused by danazol or GnRH agonists, determine a regression of the disease. Moreover, endometriosis may relapse in postmenopausal women who have been treated with ET. These clinical observations suggest a role for E in the pathogenesis of endometriosis (2).

Ectopic endometriotic implants contain estrogen receptors (ER) as shown by hormone–ligand-binding assays, immunohistochemistry, reverse transcription-polymerase chain reaction (RT-PCR), and by in-situ hybridization (3). The implants also express aromatase cytochrome P450, an enzyme that catalyses the conversion of androgens to E. Therefore, the E produced in situ, added to that already present in the circulation, increase the local concentration of this hormone, thus stimulating the growth of tissue mediated by ERs.

The ERs are nuclear receptors existing in two isoforms, ERα and ERβ, exhibiting an E-binding domain and a DNA-binding domain. After binding to ligands, these receptors act as transcriptional factors that up-regulate or down-regulate gene expression by interacting with regulatory regions of target genes. Recent studies have postulated that allelic variants (polymorphisms) of ER genes may be responsible for their action as modulators of the estrogenic response, with a potential impact on E-dependent disorders such as endometriosis (4, 5).

The aim of the present study was to evaluate a possible correlation between ER gene polymorphisms and both clinical and prognostic indices of recurrent endometriosis. The study was approved by the Institutional Review Board of the Academic Health Center of Siena, and informed consent was obtained from each participant. A total of 61 women (age range between 19 and 49 years) with a laparoscopic and histological diagnosis of endometriosis were recruited. There were a total of 22 (36%) women with one or more pregnancy and 39 (64%) nulliparas. Serum Ca-125 levels were higher than normal values in 34 (55.7%) patients.

The extent of the disease was evaluated according to the revised American Fertility Society classification (6). All the women were in reproductive age, and the following parameters were evaluated: symptoms (dyspareunia, dysmenorrhea, and pelvic pain), serum Ca-125 levels, parity, use of drug therapy, and recurrence at a second-look laparoscopy. These parameters were correlated with gene polymorphisms for ERα (by use of *Pvu*II and *Xba*I), and for ERβ (by use of *Alu*I restriction enzymes). Fisher's exact test was used to compare ERα and ERβ polymorphism frequencies between patients and controls. A $P<.05$ was accepted as the value of significance. The symptoms of endometriosis (dyspareunia, dysmenorrhea, and pelvic pain), serum Ca-125 levels, parity, presence or absence of drug therapy did not correlate with the ER gene polymorphisms.

When we considered the group of patients who had a recurrence of endometriosis (n = 13), ERα *Pvu*II polymorphisms showed a frequency of PP, Pp, and pp genotypes of 54%, 46%, and 0%, respectively. For the ERα *Xba*I polymorphisms, the frequency of XX, Xx, and xx genotypes was 46%, 46%, and 8%, respectively. As for ERβ *Alu*I polymorphisms, the frequency of AA, Aa, and aa was 23%, 69%, and 8%, respectively (Table 1).

Received December 7, 2004; revised and accepted August 25, 2005.
Reprint requests: Felice Petraglia, M.D., University of Siena, Policlinico S. Maria alle Scotte, Viale Bracci, 53100 Siena, Italy (FAX: 39-0577-233454; E-mail: petraglia@unisi.it).

0015-0282/06/$32.00
doi:10.1016/j.fertnstert.2005.08.038

TABLE 1

Endometriosis outcome in relation to ERα and ERβ polymorphisms.

ERα genotype	Endometriosis recurrency n (%)	Controls n (%)	vs. Pp	vs. pp
PP	7 (54)	6 (13)	.02[a]	.001[a]
Pp	6 (46)	27 (56)	—	NS
pp	0 (0)	15 (31)	NS	—
			vs. Xx	**vs. xx**
XX	6 (46)	4 (8)	NS	NS
Xx	6 (46)	22 (46)	—	NS
xx	1 (8)	22 (46)	NS	—
ERβ genotype			**vs. Aa**	**vs. aa**
AA	3 (23)	21 (44)	NS	NS
Aa	9 (69)	23 (48)	—	NS
aa	1 (8)	4 (8)	NS	—

Note: A statistically significant correlation of ERα *Pvu*II gene polymorphism either in homozygous PP ($P=.001$) and in heterozygous Pp ($P=.02$) conformation with recurrent endometriosis was found. NS = *P* value not significant, calculated using Fisher's exact test.

[a] *P* value significant, calculated using Fisher's exact test.

Luisi. ER gene polymorphism and recurrent endometriosis. Fertil Steril 2006.

In those patients who had no disease recurrence, the frequency of ERα *Pvu*II polymorphisms was 13% (PP), 56% (Pp), and 31% (pp), respectively. For the ERα *Xba*I polymorphisms the frequency of XX, Xx, and xx genotypes was 8%, 46%, and 46%, respectively. For the ERβ *Alu*I polymorphisms, the frequency of AA, Aa, and aa genotypes was 44%, 48%, and 8%, respectively (Table 1).

A statistically significant correlation was found between ERα *Pvu*II gene polymorphism and the recurrence of endometriosis both in PP homozygous ($P=.001$) and Pp heterozygous ($P=.02$) genotypes, whereas this was not true for homozygous pp genotype or ERα *Xba*I/ERβ *Alu*I polymorphisms.

In recent years, the association of genetic polymorphisms of ER genes and the risk of diseases have been the subject of increasing interest. Several ERα gene polymorphisms have been reported; *Pvu*II and *Xba*I polymorphisms are the most studied. Several diseases, including breast cancer, endometrial cancer, obesity, multiple sclerosis, adenomyosis (7), leiomyomas (5, 7), bone mineral density, and endometriosis (5) have been evaluated for a possible linkage with *Pvu*II and *Xba*I polymorphisms.

Endometriosis is an E-dependent disorder; a physiologic or pharmacologically induced decrease in serum E levels is associated with a regression of this disease (2).

In the present study clinical parameters, such as staging and prognosis of endometriosis (dyspareunia, dysmenorrheal, and pelvic pain), serum Ca-125 levels, parity, use of drug therapy, did not correlate with any of the ERα or ERβ gene polymorphisms. Conversely, recurrent endometriosis was associated with P ERα genotype, whereas no significant correlation with the ERβ gene polymorphism was found. The frequency of the PP and PP ERα genotypes in patients with recurrent endometriosis was higher than in controls, suggesting that the P allele predisposes to the recurrence of endometriosis. Recent studies have postulated that polymorphisms of the ERα gene may influence its action as a modulator of the ligand E, and therefore it may be associated with modifications in the manifestations of E-dependent conditions.

Interestingly, the PP ERα genotype is associated with higher bone mineral density than that showed in the Pp or the pp genotypes (7). These findings indicate a more effective estrogenic response in PP genotype vs. the opposite homozygous pp (4, 5). This hypothesis is also supported by the finding that subjects with the PP genotype have a higher risk of premenopausal hysterectomy due to menorrhagia and fibroids when compared to Pp and pp genotypes (8).

These findings contrast with previous studies showing a lower frequency of PP genotype in women with endometriosis than in controls (4, 5), with no correlation between ERα *Xba*I/ERβ *Alu*I gene polymorphisms and the staging or prognosis of endometriosis. Moreover, in contrast with what was evidenced in our study, an association of the ERβ gene *Alu*I polymorphism with an increased risk of stage IV of endometriosis was recently observed in a Japanese population (9). These discrepancies could be due to ethnic differences in the two populations as the distribution of

ERα gene polymorphisms is very different in Asian and white populations. In addition, larger populations are needed to reinforce the hypothesis of a real association between the ERα genotype and endometriosis both from a clinical and prognostic point of view.

In conclusion, our results suggested that polymorphisms in the ERα gene might be associated with the recurrence of endometriosis, probably through an increase of the ERα receptor activity. Future studies should answer questions related both to the functionality of ERα gene variants and to the number of observations, through molecular, biological, and population studies. Understanding the interplay between genetics and environmental factors in the pathogenesis of this disease will enable us to develop preventively therapeutic targeted strategies to this severe clinical disorder.

Stefano Luisi, M.D.[a]
Letizia Galleri, Ph.D.[a]
Francesca Marini, M.D.[b]
Guido Ambrosini, M.D.[c]
Maria Luisa Brandi, M.D.[b]
Felice Petraglia, M.D.[a]
[a] *Department of Pediatrics, Gynecology and Reproductive Medicine, Chair of Obstetrics and Gynecology, University of Siena, Siena;* [b] *Department of Clinical Pathophysiology, University of Florence, Florence; and* [c] *Department of Gynecological Science Reproductive Medicine, University of Padua, Padua, Italy*

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