RESEARCH ARTICLE

.

Cytochrome P450c17α 5'-untranslated region *T/C polymorphism in endometriosis

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Abstract

Estrogen plays a role in the pathogenesis of endometriosis. The CYP17 gene codes for the cytochrome P450c17α enzyme that is involved in the estrogen biosynthesis. We aimed to investigate if CYP17 polymorphism could be used as marker to predict the susceptibility of endometriosis. Women were divided into two groups: (1) severe endometriosis (n=119); (2) non-endometriosis groups (n=128). A 169-bp fragment encompassing the T/C polymorphic site in 5'-untranslated promoter region (5'-UTR) of the CYP17 was amplified by the polymerase chain reaction, treated with restriction enzyme MspA1I, and electrophoresis. The polymorphism was divided into restriction- enzyme indigestible (T homozygote), T/C heterozygote, and digestible (C homozygote). Genotypes and allelic frequencies for this polymorphism in both groups were compared. We observed a higher but non-significant percentage of T homozygote in the endometriosis women compared with the non-endometriosis women. Proportions of T homozygote/heterozygote/C homozygote for CYP17 in both groups were: (1) 26.1/46.2/27.7% and (2) 17.2/45.3/37.5% (p- value=0.131). T allele was related with higher susceptibility of endometriosis. T and C allele frequencies in both groups were: (1) 49.2/50.8%; (2) 39.8/60.2% (p- value=0.046). Despite the CYP17* T allele appearing to be asscoiatd with a trend of increased risk of endometriosis, CYP17 5'-UTR gene polymorphism might not be a useful marker for prediction of endometriosis susceptibility.

[Hsieh Y.Y., Chang C.C., Tsai F.J., Lin C.C., Tsai C.H.2004 Cytochrome P450c17α 5'-untranslated region *T/C polymorphism in endometriosis. *J. Genet.* **83**,189–192]

Introduction

Endometriosis, a frequent estrogen-dependent disease, has been suggested to have a genetic basis for its familial tendency (Hadfield *et al.*, 1997). Endometriosis is a complex disease, which is caused by an interaction between multiple genes and the environment (Sano *et al*., 1995; Kennedy, 1998). Polymorphisms are not directly linked to a certain disease. However, polymorphisms involved in steroid hormone biosynthesis and signaling may be useful genetic biomarkers for hormone-related diseases (Dunning *et al*., 1999).

Cytochrome P450c17α gene (CYP17), the gene coding for the cytochrome P450c17α enzyme, involved in estrogen biosynthesis (Carey *et al*., 1994). CYP17 mediates both steroid 17α-hydroxylase and 17,20-lyase activities and functions at key steps in the genesis of human sex steroid hormones (Habuchi *et al*., 2000). CYP17 gene maps to chromosome 10 and contains eight exons and seven introns (Picado-Leonard and Miller, 1987). The 5'-untranslated promoter region (5'-UTR) of CYP17 contains a single-bp polymorphism T to C at 34 bp upstream from the initiation of translation (Carey *et al*., 1994), which contains recognition site for the MspA1I restriction enzyme. CYP17 polymorphism may play a crucial role in the etiology of hormone-related disease such as endometriosis.

Genetic studies of the multifactorial disease such as endometriosis are difficulty to approach due to the uncertainty of a polygenic trait. The identification of the related genes is essential for genetic diagnosis and gene therapy for genetic-associated disease. We have observed that the correlation between the endometriosis and a series of gene polymorphisms, including estrogen and androgen receptors, IL-1, IL-4, TNF, p53 and p21 polymorphisms (IL-1 -511

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Keywords. cytochrome P450c17 CYP17; endometriosis; single nucleotide polymorphism.

promoter, IL-1 exon 5) (Hsieh *et al*., 2001a,b; Hsieh *et al*., 2002; Chang *et al*., 2002). In this series, using the MspA1I restriction enzyme polymorphism in 3'-UTR of CYP17, we tried to evaluate whether the CYP17 polymorphism is a useful marker for predicting the susceptibility of endometriosis. This is the first survey in this aspect.

Patients and Methods

Pre-menopausal Taiwan Chinese women with surgically diagnosed endometriosis and non-endometriosis were included. All patients were divided into two groups: (1) severe endometriosis (n=119); (2) non-endometriosis groups (n=128). The non-endometriosis statuses were confirmed during the cesarean section or diagnostic laparoscopy. All operations were performed by two surgeons (Hsieh YY, Chang CC). This study was approved by the Ethical Committee of the China Medical University Hospital. Informed consents were signed by all women who donated their blood. There were non-significant differences between both groups in age, weight, and height.

All women accepted the peripheral blood sampling for genotype analyses. Genomic DNA was isolated from peripheral blood using Genomaker DNA extractor kit (Blossom Taiwan). About 50 ng of genomic DNA was mixed with 20 pmole of each PCR primer in a total volume of 25 ul containing 10mM 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, Foster City, U.S.A.).

The 169-bp fragment encompassing the polymorphic site in the promoter region of CYP 17 T/C was amplified by PCR using primers: 5'-CCACAAGGCAAGAGATAACA-3' and 5'-AGGGTAAGCAGCAAGAGAGC-3'. PCR amplification was performed in a programmable thermal cycler GeneAmp PCR System 2400 (Perkin Elmer). The cycling condition was set as follows: one cycle at 94°C for 5 min, 35 cycles at 94° C for 30 sec, 55°C for 30 sec, 72°C for 90 sec, and one final cycle of extension at 72°C for 7 min.
The PCR products were digested overnight with 10 units of MspA1I (New England Biolabs, Inc, Beverly, MA). When the MspA1I site was present, the 169-bp PCR fragment was divided into 102 and 67 bp by the endonuclease digestion. The genotypes were designated as "T" when the restriction site was absent, and as "C" when the restriction site was present, as defined in the other studies (Carey *et al*., 1994). PCR products were analyzed by electrophoresis on 3% agarose gel. Each allele was recognized according to its size. The polymorphism was divided into undigestable (T homozygote), T/C heterozygote, and digestable (C homozygote).

Genotypes and allelic frequencies for CYP17 T/C polymorphisms in both groups were compared. Allelic frequencies are expressed as a percentage of the total number of alleles. The SAS system with 2 and Fisher's exact tests were utilized for statistical analyses. A p-value <0.05 was considered statistically significant.

Results

Genotype proportions of different CYP17 polymorphisms in both groups were non-significantly different (Table 1). We observed a higher but non-significant percentage of T homozygote in the endometriosis women compared with the non-endometriosis women. Proportions of T homozygote/heterozygote/C homozygote for CYP17 in both groups were: (1) 26.1/46.2/27.7% and (2) 17.2/45.3/37.5%, respectively (p- value=0.131, Table 1). T allele was related with higher susceptibility of endometriosis (p- value=0.046, Table 2). T and C allele frequencies in both groups were: (1) 49.2/50.8%; (2) 39.8/60.2%, respectively.

Discussion

Cytochrome P450c17 is a key enzyme in the sex steroid synthesis (Martucci and Fishman, 1993). The enzyme has both 17α-hydroxylase and 17,20-lyase activities, which is involved in the production of estrogen (Picado-Leonard and Miller, 1987). CYP17 gene polymorphism may be related with numerous tumors, including breast cancer (Feigelson

Table 1. Frequency distribution of CYP17 polymorphism in women with and without endometriosis.

Genotype	Endometriosis n=119 (%)	Non-Endometriosis n=128 (%)	*p*-value*	odds ratio
T/T	31 (26.1)	22 (17.2)	0.131	1.000
T/C	55 (46.2)	58 (45.3)		1.486
C/C	33 (27.7)	48 (37.5)		2.050

**p*-value was calculated by £q2 test
Allelic sizes (bp) after enzyme digestion were as following:
"T" allele (169 bp, uncuttable), "C" allele (102+67 bp, cuttable)

Table 2. Allelic frequency distribution of CYP17 polymorphism in women with and without endometriosis

Allele frequencies	Endometriosis n=238 (%)	Non-Endometriosis n=256 (%)	*p*-value*	odds ratio
T	117 (49.2)	102 (39.8)	0.046	1.460
C	121 (50.8)	154 (60.2)		

*p-values were calculated by χ^2 test

et al., 1997; Bergman-Jungestrom *et al*., 1999; Young *et al*., 1999), prostate cancer (Habuchi *et al*., 2000), etc. Some investigators have demonstrated that the T allele has a more andeogenic effect on men. T allele of the CYP17 polymorphism is associated with an increased risk of prostate cancer and benign prostate hyperplasia (Habuchi *et al*., 2000). In contrast, C allele has an estrogenic effect on women. C allele is associated with an increased risk of advanced breast cancer (Feigelson *et al*., 1997), polycystic ovary syndrome (Feigelson *et al*., 1998; Haiman *et al*., 1999; Diamanti-Kandarakis *et al*., 1999), and higher levels of serum estradiol (Feigelson *et al*., 1998; Haiman *et al*., 2001).

However, some investigators indicated the non-association between CYP17 polymorphism with individual diseases, including breast cancer (Nedelcheva Kristensen *et al*., 1999; Weston *et al*., 1998; Helzlsouer *et al*., 1998; Techatraisak *et al*., 1997; Dunning *et al*., 1998), polycystic ovary (Techatraisak *et al*., 1997), prostate cancer (Lunn *et al*., 1999), and steroid hormone levels [Nedelcheva Kristensen *et al*., 1999; Weston *et al*., 1998; Helzlsouer *et al*., 1998; Techatraisak *et al*., 1997; Dunning *et al*., 1998). Nedelcheva Kristensen *et al*. (1999) demonstrated that the age at onset, tumor grade, metastases, and estrogen receptor for breast cancer were not associated with the CYP17 genotype. Haiman *et al*. (1999) demonstrated that C allele of CYP17 gene is not a strong risk factor for breast cancer. Furthermore, Haiman *et al*. (2001) demonstrated that the C allele of CYP17 was at decreased risk of endometrial cancer. These controversies may be due to the multiple enzymatic processes and interactions, different illness classification, racial, environmental and disease variation. Although the exact reason for these contradictory results remains unclear, the identical CYP17 genotype may play either a protective or a promoting role in endometriosis given different environmental and/or genetic backgrounds.

In this study, we observed a higher but non-significant percentage of T homozygote in the endometriosis women compared with the non-endometriosis women. Although we did not observe significant interactions between CYP17 genotype and endometriosis risk, we did observed a trend of association between T allele and endometriosis risk. The presence of T allele seems to present an increased risk for endometriosis. Presumably, the distinct biological condition caused by the CYP17 genotype will be among various genetic, dietary, and environmental factors regulating hormonal and non-hormonal conditions in the development of endometriosis. This polymorphism may also be in linkage disequilibrium with an unidentified functional polymorphism in CYP17 that influences the endometriosis risk.

In conclusion, genotype of CYP17 5'-UTR T/C gene polymorphism might not be a useful genetic marker for the prediction of endometriosis development. Despite the CYP17* T allele appearing to indicate a trend of increased risk of endometriosis, larger series are warranted to confirm these observations and to further examine the interaction between CYP17 genotype and endometriosis development. These findings could provide a base for the further survey of the CYP17 polymorphisms. However, the real role of the CYP17 polymorphism in endometriosis remains to be clarified. Furthermore, the role of other hormone gene polymorphisms upon endometriosis development merit further examination.

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Received 14 July 2003; in revised form 5 July 2004