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Research Article

Cytochrome P450c17α (CYP17) gene polymorphism is not associated with leiomyoma susceptibility

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Abstract

Estrogen plays a role in the pathogenesis of leiomyoma. The CYP17 gene codes for the cytochrome P450c17α enzyme, which is involved in the biosynthesis of estrogen. Our aim was to investigate if CYP17 polymorphism could be a useful marker to predict the susceptibility to leiomyoma. Our sample of female subjects was divided into two groups: (1) with leiomyoma (n = 159); (2) without leiomyoma (n = 128). A 169-bp fragment encompassing the A1/A2 polymorphic site of the CYP17 gene was amplified by polymerase chain reaction (PCR), restricted by enzyme *MspA1I* and electrophored on agarose gel. Genotypes and allelic frequencies for this polymorphism in both groups were compared. There was no significant difference between the two groups regarding the distribution of the CYP17 gene polymorphism frequencies. The A1 homozygote/heterozygote/A2 homozygote proportions for CYP17 in both groups were: (1) 17.0/46.5/36.5%, and (2) 17.2/45.3/37.5%. The proportions for alleles A1 and A2 were also comparable in the two groups. A1 and A2 allele frequencies were: 7% (40.3/59) in group 1, and 2% (39.8/60) in group 2. No significant association was observed between the risk of leiomyoma and polymorphisms of the CYP 17 gene. So, CYP17 gene polymorphism does not appear to be a useful marker for the prediction of leiomyoma susceptibility.

Key words: cytochrome P450c17, CYP17, leiomyoma, single nucleotide polymorphism.

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Introduction

Leiomyoma, the most common benign uterine neoplasm, occurs in around one-forth of women during their lifetimes (Cramer *et al.*, 1992). (Leiomyoma) It is an estrogen-dependent disease, a genetic basis having been suggested for its familial tendency (Cohen *et al.*, 1988). Leiomyoma is caused by a complex interaction between multiple genes, hormone, growth factor, cytokines, and the environment. It may result from growth and proliferation of a single smooth muscle cell (Townsend *et al.*, 1970). Recently, numerous gene polymorphisms have been reported to play a role in the development of diseases. Although they are not directly linked to a certain disease, polymorphisms involved in steroid hormone biosynthesis and signaling may be useful genetic biomarkers for hormone-related diseases (Dunning *et al.*, 1999).

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P450c17α (CYP17), the gene coding for the cytochrome P450c17α enzyme is involved in estrogen biosynthesis (Carey *et al.*, 1994), mediating both steroid 17 α-hydroxylase and 17,20-lyase activities and functions as key steps in the genesis of human sex steroid hormones (Habuchi *et al.*, 2000). The CYP17 gene maps to chromosome 10 and contains eight exons and seven introns (Picado-Leonard *et al.*, 1987). The untranslated 5' region of CYP17 contains a single-nucleotide polymorphism 34 bp upstream from the transcription start site (Carey *et al.*, 1994). A single [T(A1) to C(A2)] nucleotide change in the 5' region of CYP17 creates a recognition site for the *MspA1I* restriction enzyme. Furthermore, CYP17 polymorphism may play a crucial role in the etiology of hormone-related diseases such as leiomyoma.

Genetic studies of a multifactorial disease such as leiomyoma are difficult, due to the uncertainty of a polygenic trait. The identification of related genes is essential for genetic diagnosis and gene therapy of such diseases. We investigated the relationship between leiomyoma and a number of gene polymorphisms, including urokinase, insu-

lin growth factor, and p53 (Hsieh *et al.*, unpublished data) and observed that the urokinase gene 3'-UTR C/T polymorphism and the p53 codon 72 arginine/proline polymorphism are not useful as markers for the prediction of susceptibility to leiomyoma. In contrast, the IGF2 uncuttable homozygote (AA) is associated with higher risk of leiomyoma development. In the present study, using the *MspA1I* restriction enzyme, we tried to evaluate whether the CYP17 polymorphism is a useful marker for predicting the susceptibility to leiomyoma. To the best of our knowledge, this is the first report about such a survey.

Patients and Methods

Pre-menopausal Taiwan Chinese women with surgically diagnosed leiomyoma and without leiomyoma were included. They were divided into two groups: (1) leiomyoma (n = 159); (2) non-leiomyoma (n = 128). The leiomyoma status was confirmed by sonography and pathologic examination. All operations were performed by two surgeons (Hsieh YY, Chang CC). The study was approved by the Ethics Committee and by the Institutional Review Board of the China Medical College Hospital. Informed consents were signed by all women who donated their blood. The differences between the two groups regarding age, weight, and height were non-significant.

DNA was isolated from peripheral blood using a Genomaker DNA extractor kit (*Blossom*, Taiwan). The 169-bp fragment encompassing the polymorphic site in the promoter region of CYP 17 A1/A2 was amplified by PCR. The primers used for PCR were designed as follows:

forward, 5'-CCACAAGGCAAGAGATAACA-3';
reverse, 5'-AGGGTAAGCAGCAAGAGAGC-3'.

PCR was carried out on a 25-µL aliquot containing 50 ng of genomic DNA, 50 pmol of each primer, 125 µM deoxynucleotide triphosphates, 1 unit of *Taq* polymerase (Ampli-*Taq* Gold DNA polymerase, PE Applied Biosystems), and 1x reaction buffer supplied by the manufacturer (PE Applied Biosystems). PCR amplification was performed in a programmable thermal cycler GeneAmp PCR System 2400 (Perkin Elmer Applied Biosystems, Foster City, USA). The cycling condition for CYP17 A1/A2 gene polymorphism was set as follows: one cycle at 94 °C for 5 min, 35 cycles at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 90 s, and one final extension cycle at 72 °C for 7 min.

The PCR products were digested overnight with 10 units of *MspA1I* (New England Biolabs, Inc, Beverly, MA) and analyzed by electrophoresis on 3% agarose gel. Each allele was recognized by its size. Whenever the *MspA1I* site was present, the 169-bp PCR fragment was divided into 102 and 67 bp by the endonuclease digestion. The undigested and digested fragments denote the alleles A1 and A2, respectively (Carey *et al.*, 1994). Genotypes and allelic frequencies for CYP17 A1/A2 polymorphisms in both groups were compared. Allelic frequencies are expressed as a percentage of the total number of alleles. The SAS system with χ^2 and Fisher's exact tests were utilized for the statistical analyses. A value of p 0.05 was considered statistically significant.

Results

The difference between the two groups regarding the genotype proportions of different CYP17 gene polymorphisms were non-significant (Table I). Most of the CYP17 genotypes in both groups were A1/A2 heterozygote. The proportions of A1 homozygote/heterozygote/A2 homozygote for CYP17 were: 17.0/46.5/36.5% in group 1, and 17.2/45.3/37.5% in group 2, respectively. There was also no significant difference in CYP17 allele frequencies be-

Table I - Frequency distribution of CYP17 gene polymorphism in women with and without leiomyoma.

Genotype	Leiomyoma n= 159 (%)	Non-leiomyoma n= 128 (%)	Value of p*	Odds ratio
A1/A1	27 (17.0)	22 (17.2)	0.978	1.000
A1/A2	74 (46.5)	58 (45.3)		0.962
A2/A2	58 (36.5)	48 (37.5)		1.016

*Values of p were calculated by χ^2 test.
Allelic sizes (bp) after enzyme digestion were the following:
Allele A1 (210 bp, uncuttable); allele A2 (185+25 bp, cuttable).

Table II - Allelic frequency distribution of CYP17 gene polymorphism in women with and without leiomyoma.

Allele frequencies	Leiomyoma n= 318 (%)	Non-leiomyoma n= 256 (%)	Value of p*	Odds ratio
A1	128 (40.3)	102 (39.8)	0.989	1.017
A2	190 (59.7)	154 (60.2)		

*Values of p were calculated by χ^2 test.

tween the two groups (Table 2). The most frequent CYP17 allele in both groups was allele A2. A1 and A2 allele frequencies were: 40.3/59.7%in group 1, and 39.8/60.2% in group 2, respectively.

Discussion

Leiomyoma is the most common tumor in women. However, the factors involved in its initiation and growth remain poorly understood. Leiomyoma is a monoclonal tumor. The neoplastic transformation of myometrium into leiomyoma is likely to involve somatic mutations of the normal myometrium and complex interactions of sex steroids and local growth factors (Rein, 2000). Estrogen may exert its mitogenic effects on leiomyoma through estrogen-dependent growth factors (Friedman *et al.*, 1990).

Cytochrome P450c17α is a key enzyme in the sex steroid synthesis (Martucci and Fishman, 1993). This enzyme has both 17-α-hydroxylase and 17,20-lyase activities, and is involved in the production of estrogen (Picado-Leonard *et al.*, 1987). CYP17 gene polymorphisms may be related with numerous tumors, including breast cancer (Feigelson *et al.*, 1997; Bergman-Jungestrom *et al.*, 1999; Young *et al.*, 1999) and prostate cancer (Habuchi *et al.*, 2000). The CYP17 A1 allele polymorphism has an androgenic effect upon male individuals and is associated with an increased risk of prostate cancer and benign prostatic hyperplasia (Habuchi *et al.*, 2000). In contrast, the A2 allele has an estrogenic effect on women. The A2 allele is associated with an increased risk of breast cancer (Feigelson *et al.*, 1997), polycystic ovary syndrome (Diamanti-Kandarakis *et al.*, 1999), and increased levels of serum estradiol (Haiman *et al.*, 2001; Feigelson *et al.*, 1998).

However, some investigators observed no association between CYP17 gene polymorphism and the risk of individual diseases, including ovarian cancer (Spurdle *et al.*, 2000), polycystic ovaries (Techatraisak *et al.*, 1997), breast cancer (Nedelcheva Kristensen *et al.*, 1999; Weston *et al.*, 1998; Helzlsouer *et al.*, 1998; Dunning *et al.*, 1998; Techatraisak *et al.*, 1997), and alterations of the steroid hormone levels (Weston *et al.*, 1998; Techatraisak *et al.*, 1997). 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 the A2 allele of the CYP17 gene is not a strong risk factor for breast cancer. Furthermore, Haiman *et al.* (2001) also observed that the A2 allele of CYP17 was associated with a decreased risk of endometrial cancer.

These controversies may be due to the multiple enzymatic processes and interactions, different illness classifications, racial, environmental and disease variations. In this study, we observed that the genotype distributions and allelic frequencies of the CYP17 gene were similar between the individuals with leiomyoma and the normal population. These findings indicate that CYP17 gene polymorphism is not associated with leiomyoma development. Although the exact reason for these controversial results remains unclear, a specific CYP17 genotype may play either a protective or a promoting role in leiomyoma, given different environmental and/or genetic backgrounds.

In conclusion, no significant association was observed between leiomyoma risk and CYP 17 polymorphism. CYP17 3'-UTR A1/A2 polymorphism genotypes and alleles are not candidate genetic markers for the prediction of leiomyoma susceptibility. However, the study of larger series of patients is needed to confirm this observation.

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