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Polymorphism for Transforming Growth Factor Beta 1-509 (*TGF-B1-509*): Association with Endometriosis

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Transforming growth factor beta (TGF-B) family members are multi-functional cytokines that play a key role in cellular growth, proliferation, and differentiation. The aim of the study was to investigate whether the TGF-B1-509 *gene polymorphism could be used as a marker of susceptibility in endometriosis. Women were divided into two groups: endometriosis (*n = 150*) and non-endometriosis (*n = 159*). Polymorphisms for* TGF-B1-509 *genes were amplified by polymerase chain reaction and detected after restriction enzyme digestion. Genotypes and allelic frequencies in both groups were compared. Genotype proportions and allele frequencies of TGF-B1 gene polymorphisms differed significantly in both groups. Proportions of C homozygote, heterozygote, and T homozygote for TGF-B1 gene polymorphisms were 9.3/61.3/29.4% in the endometriosis group and 41.3/58.5/0% in the non-endometriosis group. Alleles C and T for TGF-B1 gene polymorphism were 40/60% (endometriosis) and 70.8/29.2% (non-endometriosis). Association of endometriosis with* TGF-B1-509 *gene polymorphism exists. T homozygote and T allele for TGF-B1 are associated with higher susceptibility to endometriosis.*

KEY WORDS: cytokine; endometriosis; polymorphism; transforming growth factor.

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INTRODUCTION

Endometriosis, a complex disease, is associated with immunologic and genetic changes (Akoum *et al*., 2000). Endometrial cells may synthesize cytokines and growth factors, which may modulate some of the molecular mechanisms of endometrial proliferation and differentiation (Loverro *et al*., 1999). Transforming growth factor beta (TGF-B) is a family of multifunctional cytokines, which play a central role in wound healing and fibrosis as well as cellular growth, proliferation, and differentiation (Bayat *et al*., 2002b). TGF-B also influences the processes of immune responses, fibrosis, and angiogenesis (Sugiura *et al*., 2002). TGF may inhibit natural killer activity and induce angiogenesis and proliferation of endometrial stromal cells (Loverro *et al*., 2001).

TGF-B is a multifunctional cytokine involved in pro- and anti-inflammatory pathways and is expressed in several cell types. TGF-B and its related genes play an important role in many diseases. The functional impact of the TGF-B1 gene polymorphism as well as the TGF-B1 level might contribute to pathogenesis of inflammatory disease, such as endometriosis. Genetic identification is essential for early diagnosis and genetic therapy of some genetic-associated diseases. Genetic studies of multifactorial diseases such as endometriosis are difficult to approach due to the uncertainty of a polygenic trait.

The role of TGF gene polymorphism in the development of endometriosis remains unclear. Scanty reports about the role of TGF in endometriosis have appeared. In our previous reports, we observed a positive correlation of endometriosis with IL-1 (IL-1β-511 promoter, IL-1β exon 5) and p53 polymorphisms (Hsieh *et al*., 2001a,b; Chang *et al*., 2002). We also noted the non-correlation of endometriosis with the insulin growth factor (Hsieh *et al*., 2003), IL-4, TNF, and p21 (Hsieh *et al*., 2002). In this series, using the *Dde*I restriction enzyme polymorphism in exon 509 of TGF, we tried to evaluate whether this TGF-B1 polymorphism is a useful marker for predicting susceptibility to endometriosis. This is the first survey in this respect.

PATIENTS AND METHODS

Premenopausal Taiwan Chinese women with surgically diagnosed endometriosis and non-endometriosis were included. All patients were divided into two groups: (1) moderate/severe endometriosis ($n = 150$); (2) non-endometriosis ($n = 159$). The non-endometriosis status was confirmed during cesarean section or diagnostic laparoscopy. The stages of endometriosis were evaluated according to the revised American Fertility Society classification (1985). All surgeries were performed by two surgeons (Y-Y. Hsieh and C-C. Chang). This study was approved by the Ethical Committee and institute reviewing board of the China Medical University Hospital. Informed consents were signed by all women who

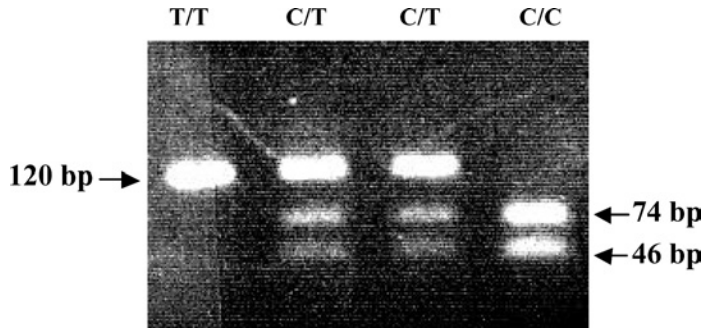

Fig. 1. Electrophoresis of different genotypes of transforming growth factor beta 1-509.

donated their blood. Age, weight, and height differences between the groups were nonsignificant.

All women accepted the use of peripheral blood sampling for genotype analysis. Genomic DNA was isolated from peripheral blood using a 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 μL containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.0 mM $MgCl_2$, 0.2 mM each deoxyribonucleotide triphosphate, and 1 unit of Amplitaq DNA polymerase (Perkin Elmer Applied Biosystems, Foster City, Calif.).

For the *Dde*I polymorphism analysis, TGF-B1 exon 9 was amplified by using two polymerase chain reaction (PCR) primers. The sequences of the 2 primers were as follows (from 5′ to 3′ end): Upstream, GGAGAGCAATTCTTACAGGTG; Downstream, TAGGAGAAGGAGGGTCTGTC. PCR conditions were as follows: 35 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s. After complete *Dde*I digestion (0.5 unit *Dde*I in 10 μL buffer for 30 min at 37°C), the product was a single fragment of 120 bp for the T allele (undigested by *Dde*I) or two fragments of 74 and 46 bp for the C allele (digested by *Dde*I).

PCR products were analyzed by electrophoresis on 3% agarose gel. Each allele was recognized according to its size (Fig. 1). Genotype and allelic frequencies for TGF-B1 polymorphism in both groups were compared. Allelic frequencies are expressed as a percentage of the total number of alleles. The SAS system with χ^2 test was utilized for statistical analyses. A $p < 0.05$ was considered statistically significant.

RESULTS

Genotype proportions and allele frequencies of *TGF-B1-509* gene polymorphisms were significantly different between the two groups. Most genotypes for

Table I. Genotypes for *TGF-B1-509* Gene Polymorphism in Women with and Without Endometriosis

Genotype[a]	Endometriosis ($n = 150$)	Non-endometriosis ($n = 159$)	p-value[b]
C/C	14 (9.3)	66 (41.5)	0.000014
C/T	92 (61.3)	93 (58.5)	
T/T	44 (29.4)	0 (0)	

Note. Percentages are shown in parentheses.
[a] Allelic size (bp) after enzyme digestion for C allele was 74 + 46 bp, cuttable; for T allele 120 bp, uncuttable.
[b] p-value was calculated by χ^2 tests.

TGF-B1 polymorphism in both groups were heterozygotes. Proportions of C homozygote, heterozygote, and T homozygote for TGF-B1 gene polymorphisms were 9.3/61.3/29.4% in the endometriosis group and 41.3/58.5/0% in the non-endometriosis group (Table I). T homozygotes *TGF-B1-509* are associated with higher susceptibility to endometriosis ($p = 0.000014$). There was no normal individual with T homozygous. Frequencies for alleles C and T for TGF-B1 gene polymorphism were 40/60% in the endometriosis group and 70.8/29.2% in the non-endometriosis group (Table II). The T allele for *TGF-B1-509* is associated with higher susceptibility to endometriosis ($p = 0.000026$).

DISCUSSION

Endometriosis, a complex immunologic disease, is related to changes of several cytokines in serum and peritoneal fluid (Pellicer *et al*., 1998). Inflammation and genetics are both prominent mechanisms in the pathogenesis of endometriosis. Endometriosis involves a complex interaction between immunoinflammatory processes, cytokine activation, and genetic factors (Vigano *et al*., 1998). Endometriosis displays features similar to malignancy, including local invasion and aggressive spread to distant organs. Numerous cytokines are associated with the

Table II. Allelic Frequencies for *TGF-B1-509* Gene Polymorphism in Women with and Without Endometriosis

Allele frequencies	Endometriosis ($n = 300$)	Non-endometriosis ($n = 318$)	p-value[a]
Allele C	120 (40)	225 (70.8)	0.000026
Allele T	180 (60)	93 (29.2)	

Note. Percentages are shown in parentheses.
[a] p-value was calculated by χ^2 tests.

molecular mechanism of endometriosis. Several growth factors, including TGF, insulin growth factor, epidermal growth factor, fibroblast growth factor, and platelet-derived growth factors, are present in the endometrium (Ferriani *et al*., 1993).

Previous studies have implicated TGF-B receptors in a variety of important hereditary clinical disorders. Transmembrane signaling by TGF-B occurs via a complex of the serine/threonine kinases TGF-B type 1 (TGF-B1), type 2 (TGF-B2), and type 3 (TGF-B3) receptors (Lucke *et al*., 2001). TGF-B1 is a multifunctional cytokine, which inhibits both development of Th1 and Th2 subsets and the Th1 proinflammatory response (Bijlsma *et al*., 2002). TGF-B1, combined with other paracrine factors, is related to the complex remodeling and differentiation processes during endometrial cycling changes (Osteen *et al*., 2002). TGF-B1 could regulate the production of urokinase plasminogen activator, which further influences endometriosis development (Guan *et al*., 2003). TGF-B1 and its receptor have important roles in the maintenance and propagation of endometriosis (Loverro *et al*., 2001).

Single nucleotide polymorphism (SNP) has been observed in various types of human cancers and is considered an important mechanism in tumorigenesis. TGF-B1 production is influenced through several SNPs in the structural gene and promoter region. The TGF-B gene, located at chromosome 19q13.1, may be a candidate locus for susceptibility to numerous diseases.

Recently, TGF-B gene polymorphisms have been reported to be associated with increased risk or severity of numerous diseases, including cervical carcinoma (Stanczuk *et al*., 2002), systemic sclerosis (Ohtsuka *et al*., 2002), rheumatoid arthritis (Sugiura *et al*., 2002), hypertension (Yamada *et al*., 2002), diabetic retinopathy (Beranek *et al*., 2002), hepatitis C (Vidigal *et al*., 2002), asthma (Pulleyn *et al*., 2001), osteoporosis (Hinke *et al*., 2001), Crohn's disease (Schulte *et al*., 2001), etc. In contrast, some investigators demonstrated the non-association of the TGF-B gene polymorphisms with individual diseases, including melanoma (Edmunds *et al*., 2002), keloid disease (Bayat *et al*., 2002a), Alzheimer's disease (Araria-Goumidi *et al*., 2002), tuberculosis (Niimi *et al*., 2002), and periodontitis (Holla *et al*., 2002). TGF-B polymorphisms do not have a strong influence on disease onset or clinical progression in sarcoidosis and tuberculosis (Araria-Goumidi *et al*., 2002).

The T homozygote and T allele, which are associated with higher production of TGF-B1, are associated with higher risk of rheumatoid arthritis (Sugiura *et al*., 2002). Cervical cell carcinoma was noted to be devoid of TGF-B, which suggested that elevated TGF-B levels could protect against cervical cancer (Stanczuk *et al*., 2002). TGF-B is a potent stimulator of collagen production by fibroblasts, which play a role in the pathogenesis of systemic sclerosis (Ohtsuka *et al*., 2002). Serum levels of TGF-B1 were higher in women with the TT genotype than in those with the CC genotype (Hinke *et al*., 2001). The CC genotype was associated with higher bone marrow density and decreased bone loss (Hinke *et al*., 2001).

In this study, we also observed that the genotype distributions and allelic frequencies for TGF-B1 were different between individuals with endometriosis and normal populations. The T homozygote and T allele for TGF-B1 were associated with higher susceptibility to endometriosis. This finding was compatible with the above reports, which demonstrated that the T allele was associated with higher risk and severity of individual diseases (Sugiura *et al*., 2002; Stanczuk *et al*., 2002; Ohtsuka *et al*., 2002; Hinke *et al*., 2001). Therefore, TGF-B1 polymorphisms may become a candidate genetic marker for susceptibility to endometriosis. The functional impact of the TGF-B1 genotype-phenotype might contribute to the pathogenesis of endometriosis.

In conclusion, endometriosis is associated with *TGF-B1-509* gene polymorphism. The T homozygote and T allele for *TGF-B1-509* are associated with higher susceptibility to endometriosis. Genotype and allele frequencies of TGF-B1 polymorphism are useful markers for the prediction of endometriosis susceptibility. This could provide a preliminary database for further surveys of the TGF-B1 polymorphisms. The real role and mechanism of the TGF-B1 polymorphism in endometriosis, however, remains to be clarified. Furthermore, the roles of other TGF subtypes and their receptors as well as other growth factors in the development of endometriosis merit further surveys.

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