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Association between endometriosis and polymorphisms in insulin-like growth factor binding protein genes in Korean women

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ABSTRACT

Objective: Genetic factors are known to be associated with the development and progression of endometriosis, but the genes related to endometriosis have not been defined. Insulin-like growth factor binding proteins (IGFBPs) are believed to be involved in the proliferation and apoptosis of cells that play an important role in the pathophysiologic mechanism of endometriosis. This study aimed to determine the association between endometriosis and polymorphisms of the IGFBP genes in Korean women.
Study design: In a case–control study, the rs1995051, rs1065780 and c.759A > G single nucleotide polymorphisms (SNPs) in the *IGFBP1* gene and the −672A > G, −202A > C and c.95C > G SNPs in the *IGFBP3* gene were analyzed in 128 women with endometriosis and 108 normal control women.
Results: The haplotype genotype composed of a combination of three *IGFBP1* gene polymorphisms was not related to endometriosis, while the haplotype genotype of the *IGFBP3* gene had a significant association with endometriosis. Women not carrying the AAG (−672A/−202A/c.95G) haplotype allele of three *IGFBP3* gene polymorphisms have a 3.19-times higher risk of endometriosis compared with women with AAG homozygotes, and this trend was found in women with advanced endometriosis but not in women with early endometriosis.
Conclusions: The AAG haplotype allele of the −672A > G, −202A > C and c.95C > G polymorphisms in the *IGFBP3* gene may be associated with advanced endometriosis in Korean women.

1. Introduction

Endometriosis is characterized by abnormal presence and growth of endometrial tissue outside the uterus [1]. Endometriosis causes several problems, such as infertility, dysmenorrhea and pelvic pain, in women of reproductive age. Although many studies have been undertaken to identify the pathogenesis of endometriosis, the mechanisms involved in the growth of ectopic endometrium remain unclear. A large body of evidence suggests that genetic factors play important roles in the development and progression of endometriosis [2,3], but the genes responsible for these processes have not been defined.

We have investigated the genes related to endometriosis in Korean women and have demonstrated that the alpha 2-Heremans Schmidt glycoprotein (AHSG) polymorphism [4], the vascular endothelial growth factor (VEGF) 405C > G polymorphism [5] and the IGF-II 820G > A polymorphism [6] might be genetic factors for endometriosis in Korean women. However, a polygenic background has been suggested in the pathophysiology of endometriosis, and therefore further studies on other candidate genes are needed.

Insulin-like growth factor (IGF) has been known to prevent apoptosis and act as a mitogen on endometrial cells [7], and is involved in angiogenesis [8]. IGF-binding proteins (IGFBPs) are believed not only to modulate the biologic action of IGF by regulating IGF bioavailability, but also to have antiproliferative and proapoptotic effects independent of IGF [9,10]. Among several IGFBPs, IGFBP1 is the most common in the human endometrium [11] and 75–90% of circulating IGFs are bound to IGFBP3 [12]. It has been demonstrated that genetic factors affect circulating IGFBP levels [13–15].

Various diseases including cancer have been reported to be associated with polymorphisms in the *IGFBP* genes [15–21]. Endometriosis is known to be a benign malignancy: similar to cancer, this disorder can be characterized by invasion and atypical growth of endometrial cells at the ectopic sites, and it may occur at distant sites, like metastasis of tumor. It has been recently reported that deregulation of the *IGFBP1* gene may be responsible for the loss of cellular homeostasis in endometriotic lesions [22].

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Table 1
PCR primers for SNPs in the *IGFBP1* and *IGFBP3* genes.

Gene	dbSNP ID	Base (amino acid) alteration	PCR primer	Annealing temperature (°C)	Fragment size (bp)
IGFBP1	rs1995051	−3211A > G	F:TTCCCAGAAATGGCTGTGTGA R: TCCATCCTGTGTGGGAGTGA VIC primer: TCGGCTGTAACTCCA FAM primer: TCGGCTGTGACTCCA		
	rs1065780	−575G > A	F: CCCCATCTCGCCTTTCCT R: CAGCCCACCCAGCAGTT VIC primer: TTGTTTTCTGCATTTGAG FAM primer: TTGTTTTCTGCGTTTGAG		
	rs4619	c.759A > G (I253M)	F: CCCTGGGTCTCCAGAGATCAG R: CGTGACAGAACATTATTTCATCTGGTTT VIC primer: ACTGCCAGATATATTTT FAM primer: CTGCCAGATGTATTTT		
IGFBP3	rs2132571	−801A > G	F: GAAGGCCGACAGGAGTTACA	60	452
	rs2132572	−672A > G	R: CCCACAAATACGCATCTGAA		
	rs2854744	−202A > C	F: CCACGAGGTACACACGAATG R: AGCCGCAGTGCTCGCATCTGG	60	459
	rs2854746	c.95C > G (A32G)	F: TTCCTGCCTGGATTCCACAGCTT R: GGCACTAGCGTTGACGCAGA	60	450

To the best of our knowledge, no research has been conducted on the association between endometriosis and single nucleotide polymorphisms (SNPs) in the *IGFBP* genes. To address this issue, we determined the prevalence of the rs1995051, rs1065780 and c.759A > G (rs4619) polymorphisms in the *IGFBP1* gene and the −801A > G (rs2132571), −672A > G (rs2132572), −202A > C (rs2854744) and c.95C > G (rs2854746) polymorphisms in the *IGFBP3* gene in women with and without endometriosis.

2. Materials and methods

2.1. Participants

One hundred and twenty-eight women with endometriosis were recruited in Seoul National University Hospital. Endometriosis was diagnosed by laparoscopy during infertility workup and was also confirmed by histology. None of the participants had a history of pelvic surgery or had received any medication associated with endometriosis. According to the revised American Fertility Society Classification [23], 73 and 55 women were classified as having early (stages I and II) and advanced (stages III and IV) endometriosis, respectively. One hundred and eight women served as controls and none of them had endometriosis based on laparoscopic findings. All controls had a medical history of tubal ligation and diagnostic laparoscopies were performed in these women to evaluate tubal length before reanastomosis. This study was approved by the Institutional Review Board of Seoul National University Hospital and written informed consent was obtained from each woman.

2.2. Extraction of genomic DNA and selection of SNPs in IGFBP genes

In accordance with the guidelines of the Declaration of Helsinki, blood samples were collected and genomic DNA was extracted using QiaAmp blood kits (Qiagen GmbH, Hilden, Germany). SNPs were selected according to the following criteria for *IGFBP* genes: SNPs associated with estrogen-dependent neoplasms, such as breast cancer in previous studies [15,18–20], non-synonymous SNPs or SNPs located in the promoter region in the SNP database (dbSNP).

2.3. Determination of IGFBP1 gene polymorphism

The three polymorphisms (rs1995051, rs1065780 and c.759A > G polymorphisms) of the *IGFBP1* gene were determined using the Taqman allelic discrimination assay. The polymorphic regions were amplified by polymerase chain reaction (PCR) with primers as shown in Table 1. The PCR was run using the TaqMan Universal Master mix and reactions were performed in a 96-well format in a total reaction volume of 5 μl using 20 ng of genomic DNA. The plates were placed in a thermal cycler (PE 9700, Applied Biosystems, Foster City, CA, USA) and heated at 50 °C for 2 min and then 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The TaqMan assay plates were transferred to a Prism 7900HT instrument (Applied Biosystems) and fluorescence data were analyzed using automated software (SDS 2.1, Applied Biosystems).

2.4. Determination of −801A > G and −672A > G polymorphisms in the IGFBP3 gene

The −801A > G and −672A > G polymorphisms were analyzed by direct DNA sequencing. With primers as shown in Table 1, these polymorphic regions were amplified by PCR. PCR products were electrophoresed through an agarose gel to verify the reaction and purified using a PCR clean-up kit (Qiagen GmbH). Sequences were determined by cycle sequencing using an ABI PRISM Bigdye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) on an automated DNA sequencer (Applied Biosystems).

2.5. Determination of −202A > C and c.95C > G polymorphisms in the IGFBP3 gene

The region comprising the −202A > C polymorphic site was amplified by PCR using specific primers. PCR was performed in 35 cycles as follows: denaturing at 94 °C for 5 min, denaturing again at 94 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 30 s. The amplified product was digested with restriction endonuclease *Alw*211 for 3 h at 37 °C and was electrophoresed through an agarose gel and visualized on an UV transilluminator.

To analyze the c.95C > G polymorphism, DNA fragments containing this polymorphic portion were amplified by PCR using the primers previously described by Morimoto et al. [14]. PCR products were digested with *Ava*I endonuclease and were electrophoresed as described above.

2.6. Statistical analyses

Statistical analyses were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). Genotype distributions among study subjects were tested for compliance with the Hardy–Weinberg equilibrium. Linkage disequilibria resulting from the non-random associations of genotypes at polymorphic sites were also assessed using a chi-square test. Haplotype analysis was performed using