Insulin-like growth factor II gene Apa I polymorphism is not associated with endometriosis susceptibility

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Abstract

Insulin-like growth factor II (IGF2) has been shown to play a role in abnormal cell growth and carcinogenesis. We investigated if the IGF2 gene Apa I polymorphism at exon 9 was associated with the susceptibility to endometriosis, analyzing 120 women with moderate/severe endometriosis and 103 controls. The genotype frequencies did not differ statistically between the endometriosis (aa = 25.4, ab = 57.4, bb = 17.2%) and control (aa = 20.8 ab = 52.8, bb = 26.4%) groups. The allele frequencies did not differ either: a = 54.1, b = 45.9% among women with endometriosis and a = 47.2, b = 52.8% in the control group. Therefore, no indication was found for an association of this polymorphism with endometriosis susceptibility.

Key words: endometriosis, insulin-like growth factor, IGF-2.

Received: November 14, 2001; Accepted: December 18, 2003.

Endometriosis is associated with a complex interaction of immuno-inflammatory processes, cytokine activation, and genetic factors. Endometrial cells synthesize cytokines and growth factors, modulating endometrial proliferation and differentiation (Loverro et al., 1999). Insulin-like growth factor-I (IGF-I) and their receptors were reported to be abnormally expressed in ovarian endometriosis, and may play a role in the pathogenesis of endometriosis. (Loverro et al., 2001). Insulin-like growth factor II (IGF-II) is a 67-amino acid mitogenic peptide, which may act as an autocrine or paracrine growth factor, enhancing and maintaining tumor growth (El-Badry et al., 1991; Kim et al., 1998). In fact, several tumors have been shown to oversecrete IGF-II, e.g. uterine leiomyosarcoma (Vu et al., 1995), ovarian cancer (Yun et al., 1996), endometrial cancer (Roy et al., 2000), cervical cancer (Douc-Rasy et al., 1996), breast carcinoma (Vu et al., 1997), and testicular tumors (Nonomura et al., 1997). Heterozygosity for IGF2 Apa I polymorphism at exon 9 was found to be lower in patients with uterine leiomyosarcoma than in healthy subjects (Vu et al., 1995).

As IGF2 gene is involved in cell growth and differentiation, we investigated whether the ApaI polymorphism (A/G) at exon 9 of this gene was associated with the disease, predicting susceptibility to endometriosis.

A group of 122 pre-menopausal Taiwan Chinese women with surgically diagnosed moderate/severe endometriosis was studied. The control group was formed by 106 women whose non-endometriosis status was confirmed during cesarean sections or diagnostic laparoscopy. All surgeries were performed by two of us (Hsieh YY, Chang CC). Informed consent was provided by the patients, and the study was approved by the Ethical Committee of the China Medical University Hospital. The two groups of women did not differ statistically for age (32.2 ± 2.6 vs. 33.4 ± 3.5 years), weight (51.5 ± 3.8 vs. 53.2 ± 4.3 kg) and height (158.2 ± 4.6 vs. 156.4 ± 3.6 cm).

Genomic DNA was isolated from peripheral blood using Genomaker DNA Extractor kit (Blossom, Taiwan). About 50 ng of genomic DNA was mixed with 20 pmol 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 MgCl2, 0.2 mM each deoxyribonucleotide triphosphate, and 1 unit of AmpliTaq DNA polymerase (Perkin Elmer Applied Biosystems, Foster City, CA, USA). For the Apa I (A/G) polymorphism analysis (Tadokoro et al., 1991), IGF2 exon 9 was amplified using the primers: upstream, 5’CTTGGAGTCTAAATTGCC3’ and downstream, 5’GCG GTACGAGCGACTGCCCAC3’. PCR conditions were as follows: 35 cycles at 95°C for 20 s, 60°C for 20 s, and
72 °C for 20 s. Complete *ApaI* digestion (1 unit *ApaI* in 10 µL buffer for 30 min at 37 °C) yielded a single fragment of 100 bp for the “a” allele (not digested by *ApaI*) or two digestion fragments of 63 and 37 bp for the “b” allele. PCR products were analyzed by electrophoresis on 3% agarose gel followed by ethidium bromide staining. Genotype and allele frequencies in the two groups were compared by χ² test.

Genotype and allele frequencies of the *IGF2* *ApaI* polymorphism are shown in Table 1. The frequencies of aa, ab and bb genotypes did not differ significantly between women with or without endometriosis, and the allele frequencies were not significantly different either. The observed genotype frequencies in both groups did not deviate significantly from Hardy-Weinberg equilibrium proportions. As previously reported by Chen et al. (2003), the frequency of both alleles was found to be high in our population, and about half of the individuals were heterozygous.

In conclusion, our investigation did not detect an association between the *IGF2* gene *ApaI* polymorphism and endometriosis in our population.

**References**


**Table 1 - Allele and genotype frequencies of the *IGF2* gene *ApaI* polymorphism in women with or without endometriosis.**

<table>
<thead>
<tr>
<th></th>
<th>Women with endometriosis n = 122</th>
<th>Women without endometriosis n = 106</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td>0.226</td>
</tr>
<tr>
<td>aa</td>
<td>31 (25.4)*</td>
<td>22 (20.8)</td>
<td></td>
</tr>
<tr>
<td>ab</td>
<td>70 (57.4)</td>
<td>56 (52.8)</td>
<td></td>
</tr>
<tr>
<td>bb</td>
<td>21 (17.2)</td>
<td>28 (26.4)</td>
<td></td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td>0.140</td>
</tr>
<tr>
<td>a</td>
<td>132 (54.1)</td>
<td>100 (47.2)</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>112 (45.9)</td>
<td>112 (52.8)</td>
<td></td>
</tr>
</tbody>
</table>

*numbers in parenthesis = percentage.*