Interleukin-2 receptor β gene C627T polymorphism in Korean women with endometriosis: a case–control study

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BACKGROUND: The purpose of this study was to investigate the potential association of the C627T polymorphism in the interleukin-2 receptor β gene (IL-2R β) with the risk of endometriosis in Korean women.

METHODS: Two hundred and thirty-seven women with surgically or histologically diagnosed endometriosis of stages III and IV were recruited for this study, and 164 patients with no evidence of endometriosis diagnosed by laparoscopy or laparotomy served as controls. The C627T polymorphism of the IL-2R β was assessed using the TaqMan allelic discrimination assay. χ² analysis was used to examine any differences in genotype distributions and allele frequencies of the IL-2R β C627T polymorphism between the endometriosis cases and the controls.

RESULTS: There was no statistically significant difference in the frequency of the IL-2R β C627T polymorphism between the endometriosis patients and the controls (28.7% C/C, 48.1% C/T and 23.2% T/T versus 29.3, 44.5 and 26.2%, respectively, P = 0.72) or in the T allele frequencies (47.3 versus 48.5%, respectively, P = 0.73). Even when the endometriosis cases were subdivided into stages III and IV, no statistically significant differences in genotype distributions or allele frequencies were observed among the three groups.

CONCLUSIONS: Contrary to the recent data reported in a Taiwanese population, our results suggest that the C627T polymorphism of the IL-2R β gene may not be associated with the risk of endometriosis in the Korean population.

Key words: endometriosis / interleukin-2 receptor β gene / C627T polymorphism / Hardy—Weinburg equilibrium / Korean women

Introduction

Endometriosis is a complex trait disease in the gynecologic area, characterized by complex interactions between genetic, immunological, hormonal and environmental components. Considering that retrograde menstruation is found in up to 90% of women, it has been generally accepted that additional factors, including deficient immunity against the retrograde endometrium during menstruation, are essential for the implantation of ectopic endometrial tissues (Halme et al., 1984). Namely, a defective immunosurveillance system is thought to be significantly involved in the development of endometriotic lesions like neoplasms (Seli and Arici, 2003).

Many studies have been published in which immune-related alterations were analyzed in terms of the pathogenesis of endometriosis, but conflicting results have been reported (Seli et al., 2003; Wu and Ho, 2003). It is well known that the pro-inflammatory cytokines such as interferon-gamma (IFN-γ) and interleukin-2 have a potential effect in the advanced stage of endometriosis (Garzetti et al., 1995).

Interleukin-2 (IL-2), binding with the specific interleukin-2 receptor (IL-2R), is one of the well-known cytokines due to the stimulating action on the proliferation and differentiation of B cells, T cells and non-specific cytotoxic cells including natural killer and lymphokine-activated killer cells (Anderson et al., 1993).

An investigation into several single-nucleotide polymorphisms (SNPs), which might affect cytokine actions on the ectopic endometrial implants, led Hsieh et al. (2005) to report that IL-2R β C627T presents different genotypic proportions and allelic frequencies between women with and without endometriosis, and that IL-12R β1
C378G and IL-18 A105C are not related to susceptibility to severe endometriosis. However, we have found that different studies often report contradictory findings with respect to SNP analysis in complex traits diseases, especially in endometriosis (Teramoto et al., 2004; Wang et al., 2004; Lee et al., 2007, 2008). Considering that there has been no further supporting evidence published of known polymorphisms in the IL-2R β gene, it is necessary to clarify whether a specific polymorphism in the IL-2R β gene is associated with the risk of endometriosis in another ethnic group. We performed this study to evaluate whether the C627T polymorphism of the IL-2R β gene is associated with the risk of endometriosis in the Korean population.

Materials and Methods

Subjects

Peripheral blood was obtained from a total of 401 patients. All subjects were of Korean origin, which represents a single ethnic trait. A total of 237 patients had surgical and histological evidence of advanced endometriosis, whereas another 164 patients without the disease served as controls. In the endometriosis group, the extent of the disease was staged according to the guidelines of the American Society for Reproductive Medicine (ASRM; Revised ASRM, 1997). One hundred and twenty-two patients were diagnosed with stage III endometriosis and 115 patients with stage IV endometriosis. None of the subjects had received intervention such as tubal ligation or reanastomosis of uterine cervix in both groups. The indications for surgery or diagnostic laparoscopy were benign ovarian tumor (n = 95), carcinoma in situ of uterine cervix (n = 34), pelvic inflammatory disease (n = 14), tubal intervention such as tubal ligation or reanastomosis (n = 11), dysmenorrhea (n = 3), suspicious adnexal mass (n = 3), pelvic pain (n = 2) and ectopic pregnancy (n = 2). Among the endometriosis group, 82 women were infertile, defined as 1 year of unprotected coitus without pregnancy, whereas 3 women in the control group were infertile, defined as 1 year of unprotected coitus without pregnancy, and/or pelvic pain, whereas nine women in the control group were infertile. One hundred and fifty-five women in the endometriosis group complained of dysmenorrhea (n = 95), carcinoma in situ (n = 34), pelvic inflammatory disease (n = 14), tubal intervention such as tubal ligation or reanastomosis (n = 11), dysmenorrhea (n = 3), suspicious adnexal mass (n = 3), pelvic pain (n = 2) and ectopic pregnancy (n = 2). Among the endometriosis group, 82 women were infertile, defined as 1 year of unprotected coitus without pregnancy, whereas 3 women in the control group were infertile. One hundred and fifty-five women in the endometriosis group complained of dysmenorrhea and/or pelvic pain, whereas nine women in the control group without ovarian tumor did.

Approval for this study was obtained from the Institutional Review Board at the Seoul National University Hospital, and informed consent was obtained from each woman. Age ranged from 19 to 34 years (28.8 ± 3.8, mean ± SD) in the endometriosis group, and from 31 to 55 years (46.2 ± 6.9) in the control group (P < 0.001).

Genomic DNA analysis

Genomic DNA was extracted from the peripheral blood with the Wizard DNA Purification Kit (Promega, Madison, WI, USA). Allelic discrimination was performed with the MGB primer/probe TaqMan assay on the ABI Prism 7000 Sequence Detection System. Each 20 μl PCR contained 9 pmol of forward primer 5'-AAGGACACCATCCTCGGGCT-3', 9 pmol of reverse primer 5'-CCGGTGTTCCTGCAGTTGAT-3', 2 pmol of C allele probe 5'(VIC)-CGCTGAGCCACGA(MGB)-3', 2 pmol of T allele probe 5'(FAM)-CCGCTGAGCCACGA(MGB)-3', 10 μl of 2 x Taqman Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and 25 ng DNA. The PCR cycling conditions consisted of one 2-min cycle at 50°C and one 10-min cycle at 95°C, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Distilled water was used as a negative PCR control for each amplification.

Statistical analysis

Genotype distributions were examined for significant departure from Hardy–Weinberg equilibrium (HWE) by a goodness-of-fit χ² test with 1 df, and the χ² test was used to examine differences in the proportions of genotypes between endometriosis cases and controls. A P-value of <0.05 was considered statistically significant.

Results

The genotypic distribution of the IL-2R β gene C627T polymorphism in the controls and the endometriosis group was in HWE.

The distribution of genotypes was not different between the endometriosis group and the control group (28.7% C/C, 48.1% C/T and 23.2% T/T versus 29.3, 44.5 and 26.2%, respectively, P = 0.72) (Table I). There was also no difference in the T allele frequencies [47.3 versus 48.5%, respectively, P = 0.73; odds ratio (OR) 0.95; 95% confidence interval (CI) 0.71–1.26]. For further analysis, the endometriosis group was divided into subgroups according to the revised ASRM staging classification (1997). We found no difference in the frequency of C627T polymorphisms between each of the endometriosis subgroups and the control group. Additionally, when the endometriosis group was divided into two subgroups according to the bilaterality of ovarian endometrioma, the distribution of genotypes and allele frequencies of the IL-2R β gene C627T polymorphism in

| Table I Genotype and allele frequencies of the IL-2R β gene 627 C/T polymorphism in endometriosis patients and controls |
|------------------|-----------|-----------|--------------|-------|--------|--------------|-------|--------|
|                  | CC        | CT        | TT          | P-value*   | C      | T          | P-value*   | OR (95% CI) |
| Endometriosis    | 68 (28.7%) | 114 (48.1%) | 55 (23.2%)  | 0.72     | 250 (52.7%) | 224 (47.3%) | 0.73     | 0.95 (0.71–1.26) |
| III              | 35 (28.7%) | 55 (45.1%)  | 32 (26.2%)  | 0.99     | 125 (51.2%) | 119 (48.8%) | 0.94     | 1.01 (0.72–1.41)  |
| IV               | 33 (28.7%) | 59 (51.3%)  | 23 (20.0%)  | 0.41     | 125 (54.3%) | 105 (45.7%) | 0.51     | 0.89 (0.63–1.25)  |
| Unilateral endometrioma | 46 (28.9%) | 73 (45.9%)  | 40 (25.2%)  | 0.96     | 165 (51.9%) | 153 (48.1%) | 0.92     | 0.98 (0.72–1.34)  |
| Bilateral endometrioma | 22 (28.2%) | 41 (52.6%)  | 15 (19.2%)  | 0.40     | 85 (54.5%)  | 71 (45.5%)  | 0.54     | 0.88 (0.60–1.30)  |
| Control          | 48 (29.3%) | 73 (44.5%)  | 43 (26.2%)  |          | 169 (51.5%) | 159 (48.5%) |          |       |

OR, odds ratio; CI, confidence interval.

*Versus control.
patients with unilateral or bilateral endometrioma did not differ from those in the control group (Table I).

**Discussion**

The purpose of this study was to evaluate whether the C627T polymorphism of the IL-2R β gene may be associated with the risk of endometriosis in the Korean population.

Recent studies have examined SNPs in several members and regulatory components of the interleukin family, including IFN-γ, IL-1 β, IL-4, IL-10, IL-1 receptor I, IL-1 receptor antagonist and IL-2R. Although the genetic contribution of the interleukin family to endometriosis has been suggested, several IL-4, IL-6 and IL-1 β polymorphisms were reported to show no association with endometriosis (Falconer et al., 2007). However, one recent study showed an association between an IL-2R β polymorphism and endometriosis in Taiwanese women (Hsieh et al., 2005).

Given that many studies for complex diseases including endometriosis fail at the replication step and that the IL-2R β C627T polymorphism analysis has only been performed in Taiwanese women, this finding should be investigated in other ethnic groups. To our knowledge, the present study is the first attempt to replicate the reported association between the C627T polymorphism of IL-2R β and advanced stage endometriosis. In this case-control study, we have shown that the C627T polymorphism in the IL-2R β gene was not associated with the risk of advanced stage endometriosis. These findings are decidedly different from the previous data reported by Hsieh et al. (2005), who showed that the non-CC genotype may have some protective function against severe endometriosis (OR 0.32, 95% CI 0.18–0.60). This inconsistency might be explained, in part, by the fact that the genotype frequencies observed in Hsieh’s study were not in HWE and did deviate from the assumption of random mating (C/C of 18, C/T of 111 and T/T of 30). The HWE violation in the controls could be a result of several factors, including non-random mating, mutations, limited population size, selection and others (Salanti et al., 2005a, b). Assessment of HWE is employed as an initial checking step for the data quality in genetic studies. In general, genotyping errors are also known to yield distortions in genotype distributions and lead to departure from HWE. It is important that control groups exhibit the HWE state in order to represent the general population when the disease prevalence is low (Li and Li, 2008). Furthermore, the discrepancies in the data reported by these two studies may reflect differences in ethnic makeup. Subtle genetic differences between ethnicities may underlie the pathogenesis of endometriosis.

Recruiting controls from the older population seems to make the genotype of our controls more homogeneously distributed by reducing the number of patients likely to develop endometriosis as much as clinically possible (Hadfield et al., 2001).

It is also noteworthy that the lack of reproducibility can be related to the sample size. Salanti et al. (2005a, b) stated that the most realistic OR (1.1–1.5) between SNPs and a complex trait disease can be achieved with the sample size over at least 1000 subjects. Given that our study does not achieve such an ideal sample size, meta-analysis with multiple studies is needed to show the true effect magnitudes in future.

In conclusion, we have shown that the C627T polymorphism of the IL-2R β gene is not associated with advanced stage endometriosis in a Korean population. To the best of our knowledge, no study has investigated the association of the IL-2R β polymorphic loci with other complex diseases. Further studies are necessary to elucidate the role of IL-2R β in the pathogenesis of endometriosis, as well as to confirm the possible association of the IL-2R β gene polymorphism with susceptibility to endometriosis.

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