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No association between HLA-DRB1 alleles and susceptibility to advanced stage endometriosis in a Korean population

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BACKGROUND: The aetiological factors of endometriosis still remain poorly understood. While there is growing evidence that genetic and immunological factors play important roles in the pathogenesis of the disease, HLA-DRB1 alleles have been reported to be associated with the risk of endometriosis in Japanese populations. This study was performed to determine whether susceptibility to advanced endometriosis is also associated with HLA-DRB1 alleles in a Korean population, which is the closest ethnic group to Japanese. METHODS: We recruited 100 Korean patients with advanced endometriosis confirmed by surgical and histolological examinations. HLA-DRB1 genotyping was carried out in two steps. Low to intermediate resolution typing was performed by PCR sequence-specific oligonucleotide hybridization method, followed by high resolution typing utilizing group-specific amplification and PCR–single strand conformation polymorphism method. Distribution of HLA-DRB1 alleles was compared with that of 800 unrelated ethnically matched individuals as well as 108 healthy female subjects. RESULTS: Genotyping revealed that the distribution of HLA-DRB1 alleles in patients with advanced endometriosis was not different from that in the two control groups. CONCLUSIONS: The findings of the present study suggest that susceptibility of advanced endometriosis is not associated with HLA-DRB1 alleles in a Korean population, which is apparently not the case in the Japanese population.

Key words: endometriosis/HLA-DRB1 alleles/human leukocyte antigen

Introduction

Endometriosis is defined as the presence of endometrial tissue outside the uterus, causing diverse diseases, including infertility, pelvic pain and dysmenorrhoea. The prevalence of endometriosis has been found to range from 2 to 18% among women who seek tubal ligations and from 5 to 50% within infertile women (Missmer and Cramer, 2003). Although endometriosis has been described since the 1800s, the aetiological factors responsible for its histogenesis and progression remain poorly understood. As retrograde menstruation has been demonstrated in up to 90% of menstruating women with patent fallopian tubes (Halme *et al.*, 1984), it has yet to be determined why endometriosis affects only a certain group of women.

Endometriosis shows heritable tendencies, with recurrence risks of 5–8% for first-degree relatives, indicating that polygenic and multifactorial aetiology is far more likely to be the cause than Mendelian inheritance (Simpson and Bischoff, 2002). There is also substantial evidence to suggest that immunological factors play a role in the pathogenesis of endometriosis (Berkkanoglu and Arici, 2003). Peritoneal macrophages are increased in total number, concentration and activational status in patients with endometriosis (Halme *et al.*, 1987). Decreased T-cell reactivity and natural killer cell-mediated cytotoxicity have also been demonstrated in patients with the disease (Dmowski *et al.*, 1981; Oosterlynck *et al.*, 1991). Endometriosis has been associated with increased immunoglobulin levels and high frequencies of both organ-specific and non-organ-specific autoantibodies resulting from polyclonal B-cell activation (Gleicher *et al.*, 1987; Confino *et al.*, 1990). Furthermore, changes in several proinflammatory chemotactant cytokines for monocytes, macrophages and granulocytes have been identified in the peritoneal fluid of women with endometriosis (Berkkanoglu and Arici, 2003).

Based upon the presence of these immune alterations and genetic predisposition, HLA association in endometriosis has been addressed in a number of studies. Recent studies in Japanese populations have demonstrated that susceptibility to endometriosis

is associated with specific HLA class I or II alleles (Ishii *et al.*, 2002a,b, 2003; Kitawaki *et al.*, 2002), whereas no deviations in HLA class I or II allele distribution were found among the Caucasian endometriosis patients compared with controls (Moen *et al.*, 1984; Simpson *et al.*, 1984; Maxwell *et al.*, 1989; Roszkowski *et al.*, 2005). Considering that the reported associations of specific HLA alleles with endometriosis in Japanese populations were weak and that the results show some inconsistencies, it is necessary to clarify whether susceptibility to endometriosis is also associated with specific HLA alleles in another close Asian ethnic group. We performed this study to determine whether susceptibility to advanced endometriosis is also associated with HLA-DRB1 alleles in a Korean population, which is the closest to the Japanese population and made up of only one ethnic group.

Materials and methods

Subjects

Peripheral blood was obtained from a total of 100 patients who had undergone diagnostic laparoscopy, pelviscopic surgery, exploratory laparotomy or transabdominal hysterectomy. All of the subjects were of Korean origin, recruited from several provinces in the South Korean region as well as Seoul, and had surgical and histological evidence of advanced endometriosis. None of the subjects had received hormone therapy during the previous 12 months. Patients having any serious chronic medical illness or autoimmune disorders were initially excluded from the present study. The extent of the disease was staged according to the guidelines of the American Society for Reproductive Medicine (1997). Forty-one patients were diagnosed as having stage III and 59 patients had stage IV endometriosis. Ages in the endometriosis group ranged from 19 to 47 years (32.7 ± 6.7, mean ± SD). The review board for human research of Seoul National University Hospital approved this project, and informed written consent was obtained from each woman.

Eight hundred healthy Koreans who registered as bone marrow donors to the Korea Marrow Donor Program from all provinces in South Korea served as controls for comparison of HLA-DR gene polymorphisms. They were healthy volunteers aged 18–40 years and identified as Koreans by names and dates of birth. In addition, we recruited 108 healthy Korean women, whose ages ranged from 34 to 71 years (50.7 ± 8.4, mean ± SD), as control subjects. These 108 controls were unrelated mothers with no personal history of autoimmune diseases from 108 Korean families previously studied for HLA class II alleles (Song *et al.*, 2002).

HLA-DRB1 typing

Genomic DNA was extracted from peripheral blood by use of QIAamp blood kit (Qiagen, Hilden, Germany). HLA-DRB1 genotyping was carried out in two steps. First, low-to-intermediate resolution typing was performed by PCR sequence-specific oligonucleotide (SSO) hybridization method using the Dynal RELI™ SSO HLA-DRB Typing kit (Roche Diagnostic System, Branchburg, NJ, USA). High resolution DRB1 typing was then carried out by group-specific amplification and PCR–single strand conformation polymorphism method with minor modification (Bannai *et al.*, 1994; Takeuchi *et al.*, 1994).

Statistical analysis

The HLA-DRB1 allele frequencies in patients with advanced endometriosis and the control groups were compared using χ^2-test, or Fisher's exact test when an expected value was less than 5. The level of significance was set at $P < 0.05$, and odds ratios (OR) with 95% confidence intervals (CI) were calculated for those comparisons showing significant *P*-values. We made corrections simply utilizing a Bonferroni correction, in which corrected *P*-value (P_c) was obtained by multiplying the probability value by the number of alleles (29 for DRB1 alleles) (Svejgaard *et al.*, 1974), as in the previous studies reporting the association between HLA alleles and endometriosis (Ishii *et al.*, 2002a,b, 2003; Kitawaki *et al.*, 2002). Power calculation was performed utilizing PASS (Power Analysis and Sample Size; NCSS, Kaysville, UT, USA) software.

Results

The frequencies of each HLA-DRB1 allele in patients with endometriosis (n = 100, 200 alleles) in the general population (n = 800, 1600 alleles) and the healthy female controls (n = 108, 216 alleles) are shown in Table I.

The incidence of HLA-DRB1*0408 in the patient group was significantly greater compared with the general control group (1 versus 0.1%, OR = 16.2, 95% CI = 1.5–178.9, P = 0.03). The association was weak and lost significance after correction for multiple comparisons. Although the incidence of HLA-DRB1*1407 in the patient group was also significantly greater in patients with endometriosis compared with the general control group (1.5 versus 0.2%, OR = 8.1, 95% CI = 1.6–40.4, P = 0.02), the association lost its significance after correction.

No significant differences were found between patients with endometriosis and the general control group with regards to the distribution of other HLA-DRB1 alleles. The frequencies of HLA-DRB1 alleles were not different at all without correction between patients with endometriosis and the healthy female controls.

Power calculation based on the numbers of patients and controls in the present study revealed that the power to detect a significant difference of allele frequency similar to the report by Ishii *et al.* (2002b) (6 versus 1.4%) was 0.94.

Discussion

The association between the HLA antigen system and susceptibility to endometriosis has yet to be elucidated. The earlier studies utilizing serological typing methods have shown that the distribution of HLA class I or II alleles is not different between patients with and without endometriosis in the Caucasian populations (Moen *et al.*, 1984; Simpson *et al.*, 1984; Maxwell *et al.*, 1989). A recent genetic study based on a reverse slot blot method also revealed that ovarian endometriosis is not associated with particular HLA-DRB1 alleles in a Polish population (Roszkowski *et al.*, 2005). However, serological and genetic studies in Japanese populations have demonstrated possible association of specific HLA class I or II alleles with the risk of endometriosis (Ishii *et al.*, 2002a,b, 2003; Kitawaki *et al.*, 2002).

Utilizing the standard microlymphocytotoxicity technique, Ishii *et al.* (2002a) demonstrated that the frequency of HLA-Cw7 was higher in patients with advanced stage endometriosis compared with the control groups (P_c = 0.049). They also found that the prevalences of the HLA-DRB1*1403 and HLA-DQB1*0301 alleles were significantly greater in patients with

Table I. Distribution of the HLA-DRB1 alleles among the Korean patients with advanced endometriosis and control groups

HLA-DRB1 allele	Patients (200 alleles)		General control group (1600 alleles)		Healthy female control group (216 alleles)	
	n	%	*n*	%	*n*	%
*0101	13	6.5	104	6.5	19	8.8
*0301	2	1.0	36	2.3	3	1.4
*0401	1	0.5	13	0.8	0	0
*0403	9	4.5	37	2.3	4	1.9
*0404	3	1.5	21	1.3	1	0.5
*0405	15	7.5	122	7.6	21	9.7
*0406	15	7.5	98	6.1	12	5.6
*0407	0	0	3	0.2	2	0.9
*0408	2	1.0[a]	1	0.1[a]	0	0
*0410	0	0	18	1.1	2	0.9
*0701	11	5.5	103	6.4	15	6.9
*0802	3	1.5	47	2.9	4	1.9
*0803	19	9.5	111	6.9	14	6.5
*0901	19	9.5	171	10.7	18	8.3
*1001	2	1.0	19	1.2	7	3.2
*1101	10	5.0	52	3.3	4	1.9
*1104	1	0.5	0	0.0	0	0
*1111	0	0	1	0.1	0	0
*1201	7	3.5	75	4.7	10	4.6
*1202	9	4.5	48	3.0	9	4.2
*1301	3	1.5	37	2.3	4	1.9
*1302	18	9.0	152	9.5	19	8.8
*1339	0	0	1	0.1	0	0
*1401	6	3.0	50	3.1	7	3.2
*1402	0	0	1	0.1	0	0
*1403	1	0.5	13	0.8	4	1.9
*1404	0	0	1	0.1	0	0
*1405	7	3.5	51	3.2	10	4.6
*1406	2	1.0	13	0.8	0	0
*1407	3	1.5[b]	3	0.2[b]	0	0
*1412	0	0	3	0.2	0	0
*1501	14	7.0	129	8.1	25	11.6
*1502	4	2.0	52	3.3	2	0.9
*1602	1	0.5	14	0.9	0	0

[a]Odds ratio (OR) 16.2; 95% confidence interval (CI) 1.5–178.9; $P = 0.03$; corrected P (P_c) not significant.
[b]OR 8.1; 95% CI 1.6–40.4; $P = 0.02$; P_c not significant.

advanced stage endometriosis ($P_c = 0.049$ and 0.049, respectively) using PCR–restriction fragment length polymorphism analyses (2002b, 2003). Investigating the frequencies of HLA-A, -B, -C and -DRB1 in Japanese patients with endometriosis and healthy control women, Kitawaki *et al.* (2002) reported that significant positive association with endometriosis was observed for HLA-B7 ($P_c = 0.044$) and HLA-Cw0702 ($P_c = 0.040$).

As the Japanese studies reporting the possible association show some inconsistencies with weak association after correction, it is necessary to clarify whether the specific HLA alleles are significantly associated with susceptibility to endometriosis in another close East Asian ethnic group. In East Asian populations, extensive family studies on HLA alleles have been carried out for the Japanese, Northern Han Chinese, and Korean populations (An *et al.*, 1992; Tokunaga and Juji, 1992; Tokunaga *et al.*, 1996; Park *et al.*, 1998). Imanish *et al.* (1992) reported that most of the HLA allele frequencies were quite similar between Koreans and Japanese. Analysing HLA haplotypes in 107 Korean families, Park *et al.* (1998) have revealed that Koreans are the closest to Japanese, despite showing a higher degree of polymorphism in the distribution of HLA haplotypes compared to Japanese.

We analysed the distribution of HLA-DRB1 alleles in patients with surgical and histological evidence of advanced endometriosis in a Korean population and found that the distribution of HLA-DRB1 alleles in patients with the disease was not different from that of 800 unrelated ethnically matched individuals as well as 108 healthy female subjects. Whereas these findings are consistent with the previous reports on the Caucasian populations, the present study failed to replicate the original reported association between specific HLA-DR alleles and susceptibility to endometriosis in the Japanese populations. Although it is very difficult to understand the possible reasons for the inconsistencies of our results with those of Japanese populations, the following factors could be addressed for explaining the discrepancies.

Despite striking similarity, distribution of HLA-DRB1 alleles is still different between the two close ethnic groups, and the subtle genetic difference can make it possible that genetic factors involved in the pathogenesis of endometriosis vary in these populations. Therefore, the putative gene involved in the pathogenesis of endometriosis may be distinct from the HLA-DR locus in Korean population, while it is closely linked to specific HLA-DR loci in Japanese population. Alternatively, based on the weak associations as well as the inconsistent loci

with association in the Japanese studies, the previous findings in Japanese populations might reflect spurious associations. Considering the contradictory findings of the studies on the association of susceptibility to endometriosis with a single genetic locus in Japanese populations (Kitawaki *et al.*, 2001; Wang *et al.*, 2004), as well as the sufficient power of the present study (0.94), further studies recruiting more patients are necessary to confirm the possible association.

The control group in the present study comprised 800 unrelated ethnically matched individuals and 108 healthy female subjects, in whom complete exclusion of endometriosis by surgery was not feasible. The control groups in the Japanese studies were also healthy individuals including males and females. Taking account of increased incidence of endometriosis in Asian populations (Arumugam and Templeton, 1992; Sangi-Haghpeykar and Poindexter, 1995), it is possible that recruitment of patients with asymptomatic endometriosis as control subjects might have diluted the difference between patients and controls, making the association weak or absent. Therefore, it is necessary to interpret the negative findings of the present study with an assumption that up to 18% of the female control subjects might have asymptomatic endometriosis.

As it has been demonstrated that clinical symptoms, immune status, role of growth factors and morphological criteria are notably different for stage I/II and III/IV endometriosis (Arici *et al.*, 1996; Nisolle and Donnez, 1997; Thornton *et al.*, 1997), it is necessary to evaluate whether susceptibility to mild or minimal endometriosis is associated with specific HLA-DRB1 alleles in future studies. The mean age of the healthy female subjects in the present study was higher than the endometriosis patients. However, as described by Hadfield *et al.* (2001), recruiting women from this age group has the merit of maximizing the probability that they were unaffected by endometriosis, i.e. it avoids including younger women who might develop the disease in later life.

In conclusion, the findings of the present study suggest that susceptibility of advanced endometriosis is not associated with HLA-DRB1 alleles in a Korean population. Further studies recruiting other East Asian ethnic groups could be beneficial to clarify the possible association of specific HLA-DRB1 alleles with susceptibility to endometriosis.

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