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Komiya Y, Tsukino H, Nakao H, Kuroda Y, Imai H, Katoh T.	Human glutathione S-transferase A1, T1, M1, and P1 polymorphisms and susceptibility to prostate cancer in the Japanese population.	J Cancer Res Clin Oncol	131	283-242	2005
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Association between endometriosis and genetic polymorphisms of the estradiol-synthesizing enzyme genes *HSD17B1* and *CYP19*

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BACKGROUND: Endometriosis, an estrogen-dependent disease, is believed to be influenced by multiple genetic and environmental factors. Here, we evaluated whether the risk and severity of endometriosis are associated with polymorphisms in estradiol-synthesizing enzyme genes: the Ser312Gly polymorphism in 17-beta-hydroxysteroid dehydrogenase type 1 (*HSD17B1*) and the Arg264Cys polymorphism in cytochrome P450, subfamily XIX (*CYP19*). METHODS: All participants underwent diagnostic laparoscopy, and the stage of endometriosis was determined according to the Revised American Fertility Society classification. Of the 138 women enrolled, 59 had no endometriosis, 21 had stage I, 10 had stage II, 23 had stage III and 25 had stage IV. SNPs were discriminated by allele-specific oligonucleotide hybridization. RESULTS: Individuals having at least one A-allele (A/G or A/A genotype) of *HSD17B1* showed a significantly increased risk of endometriosis (A/G genotype: adjusted OR, 3.06; 95%CI 1.21–7.74; A/A genotype: adjusted OR, 3.02; 95%CI 1.08–8.43). There was a significant trend associating A/G + A/A genotypes with severity of endometriosis (*P* for trend <0.01). No statistically significant association was found for the CYP19 polymorphism. CONCLUSIONS: Evidence for association between the Ser312Gly polymorphism in *HSD17B1* and endometriosis was found in a Japanese population. The A-allele of *HSD17B1* appears to confer higher risk for endometriosis.

Key words: CYP19/endometriosis/estrogen synthesis/genetic polymorphism/HSD17B1

Introduction

Endometriosis, one of the most common causes of female infertility and chronic pelvic pain, is defined as the presence of endometrial tissue outside the uterus. Three predominant theories have been proposed for the etiology of this disease: Mullerian remnants, metaplasia and direct implantation of endometrial cells (El-Mahgoub and Yaseen, 1980; Murphy *et al.*, 1986; Fujii *et al.*, 1991). Although the exact prevalence is still not known, endometriosis affects up to 5–10% of women of reproductive age (Wheeler *et al.*, 1989). The prevalence of endometriosis is as high as 20–50% in infertile women (Strathy *et al.*, 1982; Rawson *et al.*, 1991).

Endometriosis is regarded as a complex trait, in which genetic and environmental factors contribute to the disease phenotype (Kennedy *et al.*, 1998). A variety of factors affect the development of endometriosis, including hormonal status and genetic factors. For example, women with shorter intervals between menstruation and longer duration of menses are at higher risk for endometriosis (Vercellini *et al.*, 1997). The risk of endometriosis is seven times higher if a first-degree relative has been affected by endometriosis (Simpson *et al.*, 1980). However, the interaction between genetic susceptibility and environmental factors is not yet adequately understood.

The development of endometriosis is estrogen-dependent. Endometrial implants contain estrogen and progesterone receptors (Lessey *et al.*, 1989) and respond to ovarian hormonal changes, causing local bleeding, inflammation and formation of adhesions. The three main estrogens are estradiol, estrone and estriol. Estradiol, the most active form, is produced either from estrone via 17-β-hydroxysteroid dehydrogenase type 1 (HSD17B1) or from testosterone via cytochrome P450, subfamily XIX (CYP19, aromatase) (Mitrunen and Hirvonen, 2003).

Current evidence indicates that polymorphisms in genes of drug-metabolizing enzymes can affect phenotypic metabolic

variations. The *HSD17B1* gene is located in chromosome 17q12 and has a polymorphism consisting of an A to G substitution in exon 6, resulting in an amino acid change of Ser312Gly (Puranen *et al.*, 1994). The *CYP19* gene, located in chromosome 15q21, has a polymorphism consisting of C to T substitution in exon 7, resulting in an amino acid change of Arg264Cys (Toda *et al.*, 1990).

Genetic polymorphisms involved in estrogen synthesis and metabolism may play an important role in the variation of endometriosis among individuals by altering local estrogen production or circulating levels of estrogen. Here, we evaluate whether the Ser312Gly polymorphism in *HSD17B1* and Arg264Cys polymorphism in *CYP19* are associated with the risk and severity of endometriosis. A case-control study was conducted on these two polymorphisms in patients with different stages of endometriosis and controls.

Materials and methods

The protocol for the study was approved by the Institutional Review Board of University of Miyazaki, The Jikei University School of Medicine and National Cancer Center. All subjects gave their written informed consent before the laparoscopic examination.

Study participants

This study was a part of a case–control study of endometriosis. During the years 1999–2000, 139 women were recruited at the Department of Obstetrics and Gynecology, The Jikei University School of Medicine Hospital. Participants were patients between the ages of 20 and 45 who had complained of infertility and attended the hospital. The study protocol excluded all women from the study who had ever given birth or lactated. Of the 139 women recruited, only one was excluded from subsequent analysis because a DNA sample was not available.

All participants underwent diagnostic laparoscopy, and the stage of endometriosis was determined according to the Revised American Fertility Society classification (r-AFS) (American Fertility Society, 1985). Of the 138 women enrolled, 59 women had no endometriosis, 21 had stage I, 10 had stage II, 23 had stage III and 25 had stage IV.

Cases and controls were similar in several confounding factors. Risk factors for endometriosis include age, shorter menstrual cycles and longer duration of menstrual flow (Vessey *et al.*, 1993; Eskenazi and Warner, 1997). The mean age of the cases was 32.4 years and 33.1 in the controls ($P = 0.35$). No significant difference was observed in the duration of menstruation. However, there was a significant trend towards cases having shorter menstrual cycles compared to the controls (28.8 days for cases and 30.7 for controls, $P = 0.03$).

Genotyping

Blood samples were obtained before the laparoscopic examination. Genomic DNA samples were extracted from peripheral white blood cells by using a DNA Extractor WB Kit (Wako, Osaka, Japan).

A 67 bp fragment in *HSD17B1*, including an SNP site located at 27 bases from the 5′ end, was amplified using sense (5′-CTGGGGC-AGAGGACGAGG) and biotin-labeled antisense (5′-GCGGCCGG-AGGATCG) primers. A 56 bp fragment of *CYP19*, including an SNP site located at 31 bases from the 5′ end, was amplified using biotin-labeled sense (5′-GCCATAGAAGTTCTGATAGCAG) and antisense (5′-AGTTTCTCTTCTGTGGAAATCCT) primers. PCR amplifications were performed using a TPC-200 thermal cycler (MJ Research Inc., Watertown, MA) in a total reaction volume of 25 μl containing 20 ng of DNA sample, 0.6 U AmpliTaq DNA polymerase (Applied Biosystems), 0.25 mM dNTPs, 0.2 μM primers and PCR buffer [1 × GC buffer II (Takara Bio Inc., Otsu, Japan) for *HSD17B1* and 1 × GeneAmp PCR buffer (Applied Biosystems) for *CYP19*]. The amplification protocol comprised initial denaturation at 95 °C for 5 min, then 35 cycles of denaturation at 95 °C for 15 s and annealing at 55 °C for 30 s.

SNP discriminations were conducted in a manner similar to that described previously (Maruyama *et al.*, 2004) with details as follows, based on allele-specific oligonucleotide hybridization using bio-nano magnetite particles (Takeyama *et al.*, 2000; Matsunaga *et al.*, 2001). Cy3- and Cy5-labeled detection probes were designed for each SNP as follows: Cy3-labeled *HSD17B1* A-allele detection probe (5′-CCGGGCGCAGTGCGGTG), Cy5-labeled *HSD17B1* G-allele detection probe (5′-CCGGGCGCGGTGCGGTG), Cy3-labeled *CYP19* T-allele detection probe (5′-AATCCTGCATCTT-TTTT) and Cy5-labeled *CYP19* C-allele detection probe (5′-AAAT-CCTGCGTCTTTTTT). All the following experiments were performed using the semi-automated SNP detection processor (Tanaka *et al.*, 2003).

Biotinylated PCR product (12.5 μl) and streptavidin-immobilized bio-nano magnetic particles (25 μg/12.5 μl), which were prepared according to the method described by Yoshino *et al.* (2002), were mixed and incubated for 15 min at room temperature for capturing the PCR products on the magnetic particles. PCR products were denatured into single strands by alkali treatment (10 mM NaOH). After neutralization of the mixture by neutralization buffer (100 mM Tris–HCl pH 7.5, 3 mM EDTA, 0.1% BSA), 25 μl of hybridization buffer (1 M tetramethylammonium chloride, 37.5 mM Tris–HCl pH7.5 and 3 mM EDTA) containing 12.5 pmoles of Cy3-labeled and Cy5-labeled detection probes was added to the PCR products captured on magnetic particles.

The mixture was rapidly heated up to 70 °C, and then cooled slowly to 25 °C over 10 min to allow hybridization of the detection probes and biotinylated PCR products. The optimum temperature for dissociating single-base mismatched probes was determined by analysis of dissociation curves. The mixture was heated to the optimum temperature: 56 °C for *HSD17B1* or 54 °C for CYP19, and the detection probes dissociated were removed at this temperature using an automated SNP detection processor (Maruyama *et al.*, 2004). Finally, the mixture was heated to 80 °C to liberate the hybridized detection probe into the supernatant.

The fluorescence intensity of the liberated detection probe was measured at Ex: 540 nm, Em: 570 nm for Cy3 and Ex: 645 nm, Em: 675 nm for Cy5 by a microplate reader (BMG Labtech, Offenburg, Germany), respectively. The samples were classified into three distinct categories according to the signal ratio of Cy5/Cy3: (i) those with values >2, representing samples with the homozygous G/G genotype in *HSD17B1* or the homozygous C/C genotype in *CYP19*; (ii) values <0.5, representing samples with the homozygous A/A genotype in *HSD17B1* or the homozygous T/T genotype in *CYP19*; (iii) intermediate values, representing samples with the heterozygous genotype.

Statistical analysis

To estimate the risk of endometriosis, crude odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated for subgroups with the different genotypes with respect to the Ser312Gly polymorphism in *HSD17B1* and the Arg264Cys polymorphism in *CYP19*. ORs were adjusted for three endometriosis risk factors: age, menstrual cycle and duration of menstruation, using multiple logistic

regression analysis by SPSS for Windows software (version 11.0.1J, SPSS Japan, Tokyo, Japan).

The Wilcoxon rank-sum test for trend was also used to estimate the associations between the two polymorphisms and stage of endometriosis (Goodman *et al.*, 1954). All statistical tests were based on two-tailed probability. $P < 0.05$ was considered statistically significant.

Results

Of the 138 DNA samples, 10 could not be reliably genotyped and were excluded from subsequent statistical analysis (six subjects for *HSD17B1*, four subjects for *CYP19*). The observed genotype distributions were in Hardy–Weinberg equilibrium in the control subjects.

Test for association with risk of endometriosis

Table I shows the genotypic and allelic distributions of *HSD17B1* and *CYP19* polymorphisms. Individuals with at least one *HSD17B1* A-allele (A/G or A/A genotype) showed a significantly increased risk of endometriosis (A/G genotype: adjusted OR, 3.06; 95%CI 1.21–7.74; A/A genotype: adjusted OR, 3.02; 95%CI 1.08–8.43). A similar result was obtained in comparison of the combined A/A + A/G genotypes with the G/G genotype (adjusted OR, 3.05; 95%CI 1.30–7.14). No significant association was observed between the Arg264Cys polymorphism in *CYP19* and risk of endometriosis (C/T genotype: adjusted OR, 1.35; 95%CI 0.62–2.95; T/T genotype: adjusted OR, 0.41; 95%CI 0.09–1.85).

Test for association with severity of endometriosis

To evaluate whether the Ser312Gly polymorphism in *HSD17B1* and the Arg264Cys polymorphism in *CYP19* are associated with the severity of endometriosis, participants were categorized into three groups according to r-AFS classification: controls, stage I–II and stage III–IV (Table II). There was a significant trend between the combined A/G + A/A genotypes and stage of endometriosis (P for trend <0.01). No statistically significant association was found between the Arg264Cys polymorphism in *CYP19* and stage of endometriosis (data not shown).

Discussion

In the present study, we evaluated whether two polymorphisms in estradiol-synthesizing enzyme genes are associated with the risk and severity of endometriosis. The results of this study suggested that the Ser312Gly polymorphism in *HSD17B1* is associated with both risk and severity of endometriosis, while no associations were found for the Arg264Cys polymorphism in *CYP19*.

In this study, we applied strict clinical criteria for the definition of cases and controls. One third of women with endometriosis are asymptomatic, and laparoscopy or laparotomy is indispensable for the diagnosis of endometriosis. All participants in the present study underwent diagnostic laparoscopy and were diagnosed according to the r-AFS classification. In addition, bias from confounding variables was minimized by adjusting ORs for the risk and stage of

Table I. Genotypic and allelic distribution of HSD17B1 and CYP19 polymorphisms

Polymorphisms	Cases *n* (%)		Controls *n* (%)		Crude OR (95%CI)	Adjusted OR[a] (95%CI)
	Genotype	Allele	Genotype	Allele		
HSD17B1 Ser312Gly						
G/G	13 (17.3)	G allele: 66 (44.0)	22 (38.6)	G allele: 65 (57.0)	1	1
A/G	40 (53.3)		21 (36.8)		**3.22** (1.36–7.66)**	**3.06* (1.21–7.74)**
A/A	22 (29.3)	A allele: 84 (56.0)	14 (24.6)	A allele: 49 (43.0)	**2.66* (1.02–6.94)**	**3.02* (1.08–8.43)**
A/G, A/A	62 (82.7)		35 (61.4)		**3.00** (1.35–6.68)**	**3.05** (1.30–7.14)**
CYP19 Arg264Cys						
C/C	35 (46.0)	C allele: 107 (70.4)	29 (50.0)	C allele: 81 (69.8)	1	1
C/T	37 (48.7)		23 (39.7)		1.33 (0.65–2.73)	1.35 (0.62–2.95)
T/T	4 (5.3)	T allele: 45 (29.6)	6 (10.3)	T allele: 35 (30.2)	0.56 (0.14–2.15)	0.41 (0.09–1.85)
C/T, T/T	41 (54.0)		29 (50.0)		1.17 (0.59–2.32)	1.13 (0.54–2.36)

[a]ORs adjusted for age and menstrual characteristics.
$^*P < 0.05$.
$^{**}P < 0.01$.

Table II. Severity of endometriosis associated with HSD17B1 polymorphism

Endometriosis	G/G genotype	A/G, A/A genotype	Crude OR (95%CI)	Adjusted OR[a] (95%CI)
Controls				
n (%)	22 (39.7)	35 (60.3)	1	1
Stage I-II				
n (%)	7 (23.3)	23 (76.7)	2.07 (0.76–5.62)	2.07 (0.69–6.20)
Stage III-IV				
n (%)	6 (13.3)	39 (86.7)	**4.08** (1.49–11.22)**	**3.99** (1.41–11.26)**

[a]ORs adjusted for age and menstrual characteristics.
P for trend <0.01.
$^{**}P < 0.01$.

endometriosis for variables known to affect endometriosis: age, menstrual cycle and duration of menstrual flow. Detailed questionnaires were designed to determine patients' menstrual cycle and duration of menstruation. The questionnaires were administered by a trained interviewer before the laparoscopic examination to minimize recall bias.

Retrograde menstruation is thought to be one of the main causes of endometriosis. However, retrograde menstruation is a common phenomenon (Kruitwagen *et al.*, 1991), and not all the women of reproductive age are affected by endometriosis. In short, women with endometriosis can be considered to have defects in the regulation of endometrial proliferation: (i) a tendency to proliferate endometrial tissue, and (ii) impaired clearance of abnormal endometrial tissue (Vinatier *et al.*, 2001).

Development of endometriosis is estrogen-dependent, and several features of this disease can be explained on the basis of overproduction of estrogen. Current therapy consists of hormone treatments aimed at lowering circulating estrogen. Genetic polymorphisms in the estrogen-synthesis or estrogen-metabolizing enzymes may cause inter-individual variation of levels and activity of circulating estrogen.

Estradiol, the most physiologically active form of estrogen, stimulates the proliferation of the endometrium during the ovulatory phase of the menstrual cycle. The *HSD17B1* genotypes found to be associated with endometriosis may confer increased activity or expression of HSD17B1 enzyme, causing increased exposure to estradiol. Our results suggest that possessing at least one A-allele of the Ser312Gly polymorphism in *HSD17B1* increases the risk of endometriosis. Furthermore, AG + AA genotypes showed a significantly increased risk for severe endometriosis (P for trend < 0.01). These findings support the idea that the Ser312Gly polymorphism in *HSD17B1* is associated with the risk and progression of endometriosis, especially in terms of the inter-individual variation of estradiol synthesis.

Breast and endometrial cancer, like endometriosis, are considered to be estrogen-dependent diseases. The results of this study are consistent with those of a previous study that examined the Ser312Gly polymorphism in *HSD17B1* and breast cancer risk. When the *HSD17B1* A-allele and *CYP17* A2-allele were considered as the high-risk alleles, the risk of advanced breast cancer among women carrying four high-risk alleles (*HSD17B1* AA and *CYP17* A2A2) was 2.21 compared with that of women who carried none (Feigelson *et al.*, 2001).

The *CYP19* gene, encoding aromatase, plays a crucial role in estradiol synthesis. However, we could not find any association between the Arg264Cys polymorphism in *CYP19* and endometriosis. A possible explanation for this negative result might be a lack of a functional effect for this polymorphism. A previous study reported that aromatase activity was not affected by the Arg264Cys polymorphism (Watanabe *et al.*, 1997).

Three major limitations must be considered when evaluating the results of this study. First is the relatively small number of subjects. Although we found statistically significant differences, the 95% confidence intervals were relatively wide, reflecting the small number of cases. The sample size was sufficient to detect odds ratios of three or larger with 80% power at the 5% level of significance. The lack of a significant association with the *CYP19* Arg264Cys polymorphism means only that we failed to detect a difference. It remains unclear whether this polymorphism affects endometriosis.

Secondly, the distribution of *HSD17B1* alleles in the control group deviated considerably from the expected Hardy–Weinberg equilibrium, a difference that was not significant ($P = 0.06$), but was on the edge of being so. The discrepancy may result from the small number of subjects or from the characteristics of the control group. The setting of this study is a hospital, and the control group is women complaining of infertility. Because of the requirement for a surgical diagnosis, the selection of a control group in case–control studies of endometriosis has been particularly difficult (Zondervan *et al.*, 2002), and it is difficult to exclude a selection bias completely. Development of non-invasive methods for diagnosis or a prospective randomized trial will minimize any sampling bias.

Lastly, endogenous estrogen levels were not measured in this study. *HSD17B1* is not expressed in normal endometrium or endometrial hyperplasia (Utsunomiya *et al.*, 2001). One possible explanation of the apparent influence of *HSD17B1* is that the *HSD17B1* A-allele increases the level of circulating estradiol. Although an *in vitro* study failed to demonstrate any change in HSD17B1 catalytic activity produced by the Ser312Gly polymorphism (Puranen *et al.*, 1994), a recent molecular epidemiological study showed that *HSD17B1* A/A genotype was associated with higher estradiol levels in lean women (Setiawan *et al.*, 2004). The functional effects of the Ser312Gly polymorphism in *HSD17B1* and the Arg264Cys polymorphism in *CYP19* have not yet been clearly established. Further information on functional changes and more epidemiologic studies will help clarify the association between these polymorphisms and endometriosis.

Many alleles have been reported to vary in frequency among different ethnic or geographic populations. In the present study, allelic frequencies of *HSD17B1* Ser312Gly polymorphism in control individuals were similar to previously reported distributions in Chinese and American populations: A 0.43 and G 0.57 in Japanese and Chinese populations, and A 0.51 and G 0.49 in an American population (Wu *et al.*, 2003; Setiawan *et al.*, 2004). On the other hand, large variations were found between Japanese and Caucasians in the *CYP19* Arg264Cys polymorphism: C 0.70 and T 0.30 in a Japanese population, versus C 0.96 and T 0.04 in a Caucasian population (Hefler *et al.*, 2004).

In summary, we demonstrated that the Ser312Gly polymorphism in *HSD17B1* was associated with the risk and severity of endometriosis in a Japanese population. The A-allele of the *HSD17B1* gene is considered to be a high-risk allele for endometriosis. However, no association was found between the Arg264Cys polymorphism in *CYP19* and endometriosis. The results of this study are not conclusive and further investigation is warranted. Endometriosis is a complex trait. The many factors contributing to the disease

phenotype make its pathophysiology very difficult to understand. Progress in the genetics of the disease, including understanding of genetic polymorphisms, will facilitate research on endometriosis.

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References

American Fertility Society (1985) Revised American Fertility Society classification of endometriosis. Fertil Steril 43,351–352.

El-Mahgoub S and Yaseen S (1980) A positive proof for the theory of coelomic metaplasia. Am J Obstet Gynecol 137,137–140.

Eskenazi B and Warner ML (1997) Epidemiology of endometriosis. Obstet Gynecol Clin North Am 24,235–258.

Feigelson HS, McKean-Cowdin R, Coetzee GA, Stram DO, Kolonel LN and Henderson BE (2001) Building a multigenic model of breast cancer susceptibility: CYP17 and HSD17B1 are two important candidates. Cancer Res 61,785–789.

Fujii S (1991) Secondary mullerian system and endometriosis. Am J Obstet Gynecol 165,219–225.

Goodman LA and Kruskal WH (1954) Measures of association for cross classifications. J Am Stat Assoc 49,732–764.

Hefler LA, Tempfer CB, Grimm C et al. (2004) Estrogen-metabolizing gene polymorphisms in the assessment of breast carcinoma risk and fibroadenoma risk in Caucasian women. Cancer 101,264–269.

Kennedy S (1998) The genetics of endometriosis. J Reprod Med 43, 263–268.

Kruitwagen RF, Poels LG, Willemsen WN, de Ronde IJ, Jap PH and Rolland R (1991) Endometrial epithelial cells in peritoneal fluid during the early follicular phase. Fertil Steril 55,297–303.

Lessey BA, Metzger DA, Haney AF and McCarty KS, Jr (1989) Immunohistochemical analysis of estrogen and progesterone receptors in endometriosis: comparison with normal endometrium during the menstrual cycle and the effect of medical therapy. Fertil Steril 51,409–415.

Maruyama K, Takeyama H, Nemoto E, Tanaka T, Yoda T and Matsunaga T (2004) Single nucleotide polymorphism detection in aldehyde dehydrogenase 2 (ALDH2) gene using bacterial magnetic particles based on dissociation curve analysis. Biotechnol Bioeng in press.

Matsunaga T, Nakayama H, Okochi M and Takeyama H (2001) Fluorescent detection of cyanobacterial DNA using bacterial magnetic particles on a MAG-microarray. Biotechnol Bioeng 73,400–405.

Mitrunen K and Hirvonen A (2003) Molecular epidemiology of sporadic breast cancer. The role of polymorphic genes involved in oestrogen biosynthesis and metabolism. Mutat Res 544,9–41.

Murphy AA, Green WR, Bobbie D, dela Cruz ZC and Rock JA (1986) Unsuspected endometriosis documented by scanning electron microscopy in visually normal peritoneum. Fertil Steril 46,522–524.

Puranen TJ, Poutanen MH, Peltoketo HE, Vihko PT and Vihko RK (1994) Site-directed mutagenesis of the putative active site of human 17 beta-hydroxysteroid dehydrogenase type 1. Biochem J 304,289–293.

Rawson JM (1991) Prevalence of endometriosis in asymptomatic women. J Reprod Med 36,513–515.

Setiawan VW, Hankinson SE, Colditz GA, Hunter DJ and De Vivo I (2004) HSD17B1 gene polymorphisms and risk of endometrial and breast cancer. Cancer Epidemiol Biomarkers Prev 13,213–219.

Simpson JL, Elias S, Malinak LR and Buttram VC Jr (1980) Heritable aspects of endometriosis I. Genetic studies. Am J Obstet Gynecol 137, 327–331.

Strathy JH, Molgaard CA, Coulam CB and Melton LJ 3rd (1982) Endometriosis and infertility: a laparoscopic study of endometriosis among fertile and infertile women. Fertil Steril 38,667–672.

Takeyama H, Tsuzuki H, Chow S, Nakayama H and Matsunaga T (2000) Discrimination between Atlantic and Pacific subspecies of northern bluefin tuna (Thunnus thynnus) by magnetic-capture hybridization using bacterial magnetic particles. Mar Biotechnol 2,309–313.

Tanaka T, Maruyama K, Yoda K, Nemoto E, Udagawa Y, Nakayama H, Takeyama H and Matsunaga T (2003) Development and evaluation of an automated workstation for single nucleotide polymorphism discrimination using bacterial magnetic particles. Biosens Bioelectron 19,325–330.

Toda K, Terashima M, Kawamoto T et al. (1990) Structural and functional characterization of human aromatase P-450 gene. Eur J Biochem 193, 559–565.

Utsunomiya H, Suzuki T, Kaneko C et al. (2001) The analyses of 17beta-hydroxysteroid dehydrogenase isozymes in human endometrial hyperplasia and carcinoma. J Clin Endocrinol Metab 86,3436–3443.

Vercellini P, De Giorgi O, Aimi G, Panazza S, Uglietti A and Crosignani PG (1997) Menstrual characteristics in women with and without endometriosis. Obstet Gynecol 90,264–268.

Vessey MP, Villard-Mackintosh L and Painter R (1993) Epidemiology of endometriosis in women attending family planning clinics. BMJ 306, 182–184.

Vinatier D, Orazi G, Cosson M and Dufour P (2001) Theories of endometriosis. Eur J Obstet Gynecol Reprod Biol 96,21–34.

Watanabe J, Harada N, Suemasu K, Higashi Y, Gotoh O and Kawajiri K (1997) Arginine-cysteine polymorphism at codon 264 of the human CYP19 gene does not affect aromatase activity. Pharmacogenetics 7, 419–424.

Wheeler JM (1989) Epidemiology of endometriosis-associated infertility. J Reprod Med 34,41–46.

Wu AH, Seow A, Arakawa K, Van Den Berg D, Lee HP and Yu MC (2003) HSD17B1 and CYP17 polymorphisms and breast cancer risk among Chinese women in Singapore. Int J Cancer 104,450–457.

Yoshino T, Tanaka T, Takeyama H and Matsunaga T (2003) Single nucleotide polymorphism genotyping of aldehyde dehydrogenase 2 gene using a single bacterial magnetic particles. Biosens Bioelectron 18,661–666.

Zondervan KT, Cardon LR and Kennedy SH (2002) What makes a good case-control study? Design issues for complex traits such as endometriosis. Hum Reprod 17,1415–1423.

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Analysis of the *AhR*, *ARNT*, and *AhRR* gene polymorphisms: genetic contribution to endometriosis susceptibility and severity

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Objective: To explore whether polymorphisms in *AhR*, *ARNT*, and *AhRR* contribute to endometriosis susceptibility and severity.
Design: Case control study.
Setting: Hospital.
Patient(s): One hundred thirty-eight Japanese women with or without endometriosis, diagnosed endoscopically.
Intervention(s): Endoscopic laparoscopy, with blood samples for genotyping obtained before the laparoscopic examination for genomic DNA extraction from peripheral leukocytes.
Main Outcome Measure(s): *AhR*, *ARNT*, and *AhRR* polymorphisms were genotyped using real-time polymerase chain reaction (PCR) analysis. Odds ratios and 95% confidence intervals were calculated for *AhR*, *ARNT*, and *AhRR* genotypes to evaluate the risk of endometriosis. Associations between these polymorphisms and stage of endometriosis were also examined.
Result(s): The C/G + G/G genotypes at codon 185 of *AhRR* showed a statistically significant association with risk of endometriosis (adjusted odds ratio, 2.53; 95% confidence interval, 1.16–5.55). Furthermore, a statistically significant trend associated the C/G + G/G genotypes with the clinical stage of endometriosis. No statistically significant association was observed between *AhR* codon 554 or *ARNT* codon 189 polymorphisms and endometriosis.
Conclusion(s): *AhRR* codon 185 polymorphism was associated with susceptibility to and severity of endometriosis in Japanese women. (Fertil Steril® 2005;84:454–8. ©2005 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, genetic polymorphisms, AhR, ARNT, AhRR

Endometriosis is a gynecologic condition that occurs in approximately 10% of women in the general population (1) and 40% of infertile women (2). The most common symptoms associated with pelvic endometriosis are dysmenorrhea, chronic pelvic pain, and infertility. Endometriosis is regarded as a complex trait in which genetic and environmental factors contribute to the disease phenotype (3). Genetic understanding of endometriosis has recently begun to progress rapidly, particularly through analysis of genetic polymorphisms. Genetic polymorphisms associated with endometriosis include drug metabolizing enzymes, growth factors, and hormone receptor genes (4–7).

The aryl hydrocarbon receptor (*AhR*) is a ligand-dependent transcription factor that regulates cell differentiation and the induction of the phase I and II drug-metabolizing enzymes (8, 9). The *AhR* signaling pathway regulates induction of CYP1A1 and CYP1B1, representative phase I drug metabolizing enzymes (10, 11). These isoforms catalyze the conversion of 17β-estradiol to 2-hydroxyestradiol or 4-hydroxyestradiol. Alterations in the *AhR* signaling pathway could affect the risk of endometriosis through altered expression of CYP1A1 and CYP1B1 or increased proliferation of endometrial cells.

The most well-known *AhR* ligands are polycyclic aromatic hydrocarbons, including 2,3,7,8-tetrachlorodibenzo-p-dioxin (12). Recently, Ohtake et al. (13) reported functional cross-talk between dioxin-activated *AhR* and estrogen receptors. Exposure to dioxins has been suggested as a risk factor for endometriosis (14), but several studies have reached different conclusions, and the issue remains controversial (15, 16).

The *AhR* nuclear translocator (*ARNT*) and the *AhR* repressor (*AhRR*) regulate *AhR* function. Ligand-bound *AhR* translocates to the nucleus, where it heterodimerizes with *ARNT*. The *AhR-ARNT* heterodimer binds to xenobiotic response element sequences and facilitates activation of target genes (17). In heterodimer formation, *AhRR* competes with *AhR*, thus down-regulating the genes regulated by *AhR* (18). Both *AhR* and *ARNT* are expressed in the female reproductive tract, and changes in their expression have been reported in specific pathologic conditions (19).

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Polymorphic sites have been identified in the coding regions of the *AhR*, *ARNT*, and *AhRR* genes, including *AhR* codon 554 in exon 10 (AGA to AAA, Arg to Lys), *ARNT* codon 189 in exon 7 (GTG to GTC, silent mutation), and *AhRR* codon 185 in exon 5 (CCC to GCC, Pro to Ala) (20–22).

Altered AhR-mediated signaling caused by polymorphisms in *AhR*, *ARNT*, and/or *AhRR* may account for individual differences in susceptibility to endometriosis. However, one previous study found no association between *AhR*, *ARNT*, and *AhRR* polymorphisms and endometriosis (23). In our study, we explored whether these polymorphisms contribute to the susceptibility to and severity of endometriosis. A case-control study was conducted in patients with different stages of endometriosis and controls.

MATERIALS AND METHODS

This study was approved by the institutional review board of the University of Miyazaki, the Jikei University School of Medicine, and the National Cancer Center. All participants gave their written informed consent before the laparoscopic examination.

Participants

From 1999 to 2000, 139 women were recruited at the Department of Obstetrics and Gynecology, Jikei University School of Medicine Hospital. The participants were patients between the ages of 20 and 45 who had presented with infertility and attended the hospital. The mean ages of cases and controls were similar (32 years for cases; 33 years for controls). None of the women had had prior empiric therapy with either progestins or gonadotropin-releasing hormone (GnRH) analogues before the laparoscopic examination. Women who had given birth or lactated were not eligible for this study. One woman was excluded because no DNA sample was available, leaving 138 women for the subsequent analysis.

All of the women underwent diagnostic laparoscopy as part of the infertility work-up. Women were classified into two groups according to the revised American Fertility Society (AFS) classification (24): endometriosis (stage I to IV) and controls (no endometriosis). For the most part, diagnosis was made by a single, trained gynecologist. The diagnosis of endometriosis was established by visual criteria during laparoscopic examination, and histologic confirmation was not always obtained. Of the 138 women enrolled, 59 had no endometriosis, 21 had stage I endometriosis, 10 had stage II, 23 had stage III, and 25 had stage IV.

Genotyping

Blood samples were obtained before the laparoscopic examination. Genomic DNA was extracted from peripheral leukocytes using a DNA Extractor WB Kit (Wako, Osaka, Japan). Genotyping was performed blinded to case control status, minimizing measurement bias.

TABLE 1

Primers and probes used for real-time PCR analysis.

Primers	Sequence
AhR codon 554	AGA to AAA, Arg to Lys
Forward primer	5′-AAAAACAGTGACTTGTACAGCATAATGA-3′
Reverse primer	5′-CTGAAGTCAACCTCACCAGAAAAAT-3′
Probe: G allele	5′-FAM-TGAAGACATCAGACACAT-MGB-3′
Probe: A allele	5′-VIC-AGACATCAAACACATGC-MGB-3′
ARNT codon 189	GTG to GTC, silent mutation
Forward primer	5′-TGCTGCCAAACCATTCAGACT-3′
Reverse primer	5′-GGAACTGAAACATTTGATCTTGGA-3′
Probe: G allele	5′-VIC-CGGAGTCAGACACATA-MGB-3′
Probe: C allele	5′-FAM-ACGGAGTCAGAGACAT-MGB-3′
AhRR codon 185	CCC to GCC, Pro to Ala
Forward primer	5′-AGACGGATGTAATGCACCAGA A-3′
Reverse primer	5′-AGAGGCAGCGATGTGTTATGG-3′
Probe: C allele	5′-FAM-TGGGCAGCCCCCCGCC-TAMRA-3′
Probe: G allele	5′-VIC-TGGGCAGGCCCCGCC-TAMRA-3′

Note: *AhR* = aryl hydrocarbon receptor; *ARNT* = *AhR* nuclear translocator; *AhRR* = *AhR* repressor; PCR = polymerase chain reaction.

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The *AhR*, *ARNT*, and *AhRR* polymorphisms were genotyped by real-time polymerase chain reaction (PCR) analysis on an ABI Prism 7000 Sequence Detection System (Applied Biosystems, Japan Ltd, Tokyo, Japan) using fluorescent-labeled probes (25). All the primers and probes were designed appropriately with Primer Express 2.0 software (Applied Biosystems). Two differentially-labeled TaqMan probes and forward and reverse primers were prepared for each reaction. Primers and probes are listed in Table 1.

Reactions were performed with 200 nM of each probe, 900 nM each of forward primer and reverse primers, 1X TaqMan Universal PCR Master Mix (Applied Biosystems), and 20 ng DNA. The PCR cycling conditions consisted of one 2-minute cycle at 50°C, and one 10-minute cycle at 95°C, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. HPLC water was used as a negative PCR control in each amplification.

Statistical Analysis

Crude odds ratios (OR) and 95% confidence intervals (95% CI) were calculated for *AhR*, *ARNT*, and *AhRR* genotypes to evaluate the association with endometriosis. Because endogenous estrogen exposure was thought to be correlated with the risk of endometriosis, the OR was adjusted for risk factors that might affect endogenous estrogen exposure: age (<35, ≥35 years), menstrual cycle (<26, 26 to 30, ≥31 days), and duration of menstruation (<7, ≥7 days) using multiple logistic regression analysis by SPSS for Windows software (version 11.0.1J; SPSS Japan, Tokyo, Japan) (26, 27). The Wilcoxon rank-sum test for trend was also used to examine the association between the *AhR*, *ARNT*, and *AhRR* polymorphisms and the stage of endometriosis (28). All statistical tests were based on two-tailed probability. $P<.05$ was considered statistically significant.

RESULTS

The genotype and allele frequencies of each polymorphism are shown in Table 2. The distributions of genotypes among controls were in Hardy-Weinberg equilibrium.

The C/G + G/G genotypes at codon 185 of *AhRR* showed a statistically significant association with risk of endometriosis compared with the C/C genotype (adjusted OR, 2.53; 95% CI, 1.16–5.55). No statistically significant association was observed between the *AhR* codon 554 or *ARNT* codon 189 polymorphism and the risk of endometriosis (Table 3).

Furthermore, we evaluated whether these polymorphisms were associated with the stage of endometriosis. There was

TABLE 2

Genotype and allele frequencies of *AhR*, *ARNT*, and *AhRR* polymorphisms.

Polymorphisms	Codons	Amino acids	Genotype frequencies: Endometriosis n (%)	Genotype frequencies: Controls n (%)	Allele frequencies: Endometriosis n (%)	Allele frequencies: Controls n (%)
AhR codon 554						
G/G	AGA/AGA	Arg/Arg	24 (30.4)	22 (37.3)	G: 83 (52.5)	G: 73 (61.9)
A/G	AAA/AGA	Arg/Lys	35 (44.3)	29 (49.1)	A: 75 (47.5)	A: 45 (38.1)
A/A	AAA/AAA	Lys/Lys	20 (25.3)	8 (13.6)		
ARNT codon 189						
G/G	GTG/GTG	Val/Val	26 (32.9)	19 (32.2)	G: 82 (58.2)	G: 64 (54.2)
C/G	GTC/GTG	Val/Val	40 (50.6)	28 (44.1)	C: 68 (41.8)	C: 54 (45.8)
C/C	GTC/GTC	Val/Val	13 (18.5)	14 (23.7)		
AhRR codon 185						
C/C	CCC/CCC	Pro/Pro	20 (25.3)	27 (45.8)	C: 87 (55.1)	C: 81 (88.6)
C/G	CCC/GCC	Pro/Ala	47 (59.5)	27 (45.8)	C: 71 (44.9)	C: 37 (31.4)
G/G	GCC/GCC	Ala/Ala	12 (15.2)	5 (8.4)		

Note: *AhR* = aryl hydrocarbon receptor; *ARNT* = *AhR* nuclear translocator; *AhRR* = *AhR* repressor.

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TABLE 3

***AhR, ARNT,* and *AhRR* polymorphisms and risk of endometriosis.**

Polymorphism	Endometriosis n (%)	Controls n (%)	Crude OR (95% CI)	Adjusted OR[a] (95% CI)
AhR codon 554				
G/G	24 (30.4)	22 (37.3)	1	1
A/G + A/A	55 (69.6)	37 (62.7)	1.36 (0.67–2.78)	1.65 (0.76–3.61)
ARNT codon 189				
G/G	26 (32.9)	19 (32.2)	1	1
C/G + C/C	53 (67.1)	40 (67.8)	0.97 (0.47–1.99)	0.86 (0.39–1.87)
AhRR codon 185				
C/C	20 (25.3)	27 (45.8)	1	1
C/G + G/G	59 (74.7)	32 (54.2)	2.49[b](1.21–5.12)	2.53[b](1.16–5.55)

[a] OR adjusted for age and menstrual characteristics.
[b] $P<.05$.

Tsuchiya. AhRR polymorphism and endometriosis. Fertil Steril 2005.

a statistically significant trend between C/G + G/G genotypes and the stage of endometriosis (P for trend, .02; Table 4). The *AhR* codon 554 and *ARNT* codon 189 polymorphisms were not associated with a higher risk of advanced clinical stage (data not shown).

DISCUSSION

In the present study, we evaluated whether polymorphisms at *AhR* codon 554, *ARNT* codon 189, and *AhRR* codon 185 contribute to the susceptibility to and severity of endometriosis. Our results indicate that the *AhRR* codon 185 polymorphism is associated with both susceptibility and severity. The genotypic distribution of this polymorphism was statistically significantly different in women with endometriosis and controls. The risk of endometriosis was approximately 2.5 times higher with the *AhRR* C/G + G/G genotype. In addition, the *AhRR* C/G + G/G genotype was more frequently observed in patients with an advanced stage endometriosis. No such association was found with the *AhR* codon 554 or *ARNT* codon 189 polymorphism. We should not, however, definitively conclude that no association exists between the *AhR* codon 554 or *ARNT* codon 189 polymorphism and susceptibility to and severity of endometriosis because our sample size was too small to allow for the detection of subtle differences.

In a previous study, Watanabe et al. (23) failed to find a relation between the *AhRR* codon 185 polymorphism and endometriosis. There may be several reasons for the discrepancy. First and most important is the definition of endometriosis. Because endometriosis is sometimes asymptomatic, it can be diagnosed only by laparoscopy or laparotomy. In the present study, all participants underwent laparoscopy, and the endometriosis and control groups were strictly defined according to the revised AFS classification. By this means, we could apply the best definition of cases and controls. In the previous study, control groups consisted primarily of asymptomatic volunteers. Without surgical diagnosis, the control population could contain a substantial number of women with undiagnosed endometriosis, thereby diluting the risk factor effects (29). Second,

TABLE 4

***AhRR* codon 185 polymorphism and severity of endometriosis.**

Clinical stage	*AhRR* C/C genotype	*AhRR* C/G + G/G genotype	Crude OR (95% CI)	Adjusted OR[a] (95% CI)
Controls, n (%)	27 (45.8)	32 (54.2)	1	1
Stage I–II, n (%)	8 (25.8)	23 (74.2)	2.43 (0.94–6.30)	1.78 (0.64–4.98)
Stage III–IV, n (%)	12 (25.0)	36 (75.0)	2.53[b](1.10–5.81)	3.17[b](1.27–7.91)

[a] OR adjusted for age and menstrual characteristics.
[b] $P<.05$.
P for trend: .02.

Tsuchiya. AhRR polymorphism and endometriosis. Fertil Steril 2005.

their study and ours differ in terms of participants. It is known that pregnancy and lactation can cause disease regression and improvements in symptoms of endometriosis (30,31). To reflect the authentic phenotype of endometriosis at the time of surgical diagnosis, our study excluded women who had given birth or lactated. Finally, considerable intraobserver and interobserver variability is reported in the revised AFS classification (32). Although this variability is a concern in any case control study of endometriosis, it does not decrease the validity or reliability of the association found.

Our study provides evidence for an association between the *AhRR* codon 185 polymorphism and endometriosis, although the exact mechanism for this effect is still unknown. One possible explanation for this association is alteration of *AhR*-mediated signaling by the polymorphism. The *AhRR* C/G + G/G genotype may facilitate proliferation of endometrial cells through the diminished down-regulation of *AhR*-mediated signaling. Because of the small sample size, ours is not a conclusive study, and the results need to be investigated further in large studies that also consider ethnic variation.

The *AhRR* codon 185 polymorphism is associated with susceptibility to and severity of endometriosis in Japanese women, but *AhR* codon 554 and *ARNT* codon 189 polymorphisms appear not to be associated with endometriosis. The pathogenesis of endometriosis is still not clearly understood, so the *AhRR* codon 185 polymorphism could be a useful genetic marker in predicting endometriosis susceptibility and severity.

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REFERENCES

1. Wheeler JM. Epidemiology of endometriosis-associated infertility. J Reprod Med 1989;34:41–6.
2. Rawson JM. Prevalence of endometriosis in asymptomatic women. J Reprod Med 1991;36:513–5.
3. Kennedy S. The genetics of endometriosis. Eur J Obstet Gynecol Reprod Biol 1999;82:129–33.
4. Zondervan K, Cardon L, Kennedy S. Development of a Web site for the genetic epidemiology of endometriosis. Fertil Steril 2002;78:777–81.
5. Arvanitis DA, Koumantakis GE, Goumenou AG, Matalliotakis IM, Koumantakis EE, Spandidos DA. CYP1A1, CYP19, and GSTM1 polymorphisms increase the risk of endometriosis. Fertil Steril 2003;79:702–9.
6. Chang CC, Hsieh YY, Tsai FJ, Tsai CH, Tsai HD, Lin CC. The proline form of p53 codon 72 polymorphism is associated with endometriosis. Fertil Steril 2002;77:43–5.
7. Georgiou I, Syrrou M, Bouba I, Dalkalitsis N, Paschopoulos M, Navrozoglou I, et al. Association of estrogen receptor gene polymorphisms with endometriosis. Fertil Steril 1999;72:164–6.
8. Elizondo G, Fernandez-Salguero P, Sheikh MS, Kim GY, Fornace AJ, Lee KS, et al. Altered cell cycle control at the G(2)/M phases in aryl hydrocarbon receptor–null embryo fibroblast. Mol Pharmacol 2000;57:1056–63.
9. Telakowski-Hopkins CA, King RG, Pickett CB. Glutathione S-transferase Ya subunit gene: identification of regulatory elements required for basal level and inducible expression. Proc Natl Acad Sci USA 1988;85:1000–4.
10. Whitlock JP Jr. Induction of cytochrome P4501A1. Annu Rev Pharmacol Toxicol 1999;39:103–25.
11. Alexander DL, Eltom SE, Jefcoate CR. Ah receptor regulation of CYP1B1 expression in primary mouse embryo-derived cells. Cancer Res 1997;57:4498–506.
12. Lucier GW, Portier CJ, Gallo MA. Receptor mechanisms and dose-response models for the effects of dioxins. Environ Health Perspect 1993;101:36–44.
13. Ohtake F, Takeyama K, Matsumoto T, Kitagawa H, Yamamoto Y, Nohara K, et al. Modulation of oestrogen receptor signalling by association with the activated dioxin receptor. Nature 2003;423:545–50.
14. Rier SE, Martin DC, Bowman RE, Dmowski WP, Becker JL. Endometriosis in rhesus monkeys (Macaca mulatta) following chronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Fundam Appl Toxicol 1993;21:433–41.
15. Pauwels A, Schepens PJ, D'Hooghe T, Delbeke L, Dhont M, Brouwer A, et al. The risk of endometriosis and exposure to dioxins and polychlorinated biphenyls: a case-control study of infertile women. Hum Reprod 2001;16:2050–5.
16. Lebel G, Dodin S, Ayotte P, Marcoux S, Ferron LA, Dewailly E. Organochlorine exposure and the risk of endometriosis. Fertil Steril 1998;69:221–8.
17. Wilson CL, Safe S. Mechanisms of ligand-induced aryl hydrocarbon receptor-mediated biochemical and toxic responses. Toxicol Pathol 1998;26:657–71.
18. Mimura J, Ema M, Sogawa K, Fujii-Kuriyama Y. Identification of a novel mechanism of regulation of Ah (dioxin) receptor function. Genes Dev 1999;13:20–5.
19. Khorram O, Garthwaite M, Golos T. Uterine and ovarian aryl hydrocarbon receptor (*AhR*) and aryl hydrocarbon receptor nuclear translocator (*ARNT*) mRNA expression in benign and malignant gynaecological conditions. Mol Hum Reprod 2002;8:75–80.
20. Kawajiri K, Watanabe J, Eguchi H, Nakachi K, Kiyohara C, Hayashi S. Polymorphisms of human Ah receptor gene are not involved in lung cancer. Pharmacogenetics 1995;5:151–8.
21. Scheel J, Hussong R, Schrenk D, Schmitz HJ. Variability of the human aryl hydrocarbon receptor nuclear translocator (*ARNT*) gene. J Hum Genet 2002;47:217–24.
22. Cauchi S, Stucker I, Cenee S, Kremers P, Beaune P, Massaad-Massade L. Structure and polymorphisms of human aryl hydrocarbon receptor repressor (*AhRR*) gene in a French population: relationship with CYP1A1 inducibility and lung cancer. Pharmacogenetics 2003;13:339–47.
23. Watanabe T, Imoto I, Kosugi Y, Fukuda Y, Mimura J, Fujii Y. Human aryl hydrocarbon receptor repressor (*AHRR*) gene: genomic structure and analysis of polymorphism in endometriosis. J Hum Genet 2001;46:342–6.
24. American Fertility Society. Revised American Fertility Society classification of endometriosis. Fertil Steril 1985;43:351–2.
25. de Kok JB, Wiegerinck ET, Giesendorf BA, Swinkels DW. Rapid genotyping of single nucleotide polymorphisms using novel minor groove binding DNA oligonucleotides (MGB probes). Hum Mutat 2002;19:554–9.
26. Vessey MP, Villard-Mackintosh L, Painter R. Epidemiology of endometriosis in women attending family planning clinics. BMJ 1993;306:182–4.
27. Eskenazi B, Warner ML. Epidemiology of endometriosis. Obstet Gynecol Clin North Am 1997;24:235–58.
28. Goodman LA, Kruskal WH. Measures of association for cross classifications. J Am Stat Assoc 1954;49:732–64.
29. Zondervan KT, Cardon LR, Kennedy SH. What makes a good case-control study? Design issues for complex traits such as endometriosis. Hum Reprod 2002;17:1415–23.
30. Schenken RS, Williams RF, Hodgen GD. Effect of pregnancy on surgically induced endometriosis in cynomolgus monkeys. Am J Obstet Gynecol 1987;157:1392–6.
31. Barragan JC, Brotons J, Ruiz JA, Acien P. Experimentally induced endometriosis in rats: effect on fertility and the effects of pregnancy and lactation on the ectopic endometrial tissue. Fertil Steril 1992;58:1215–9.
32. Hornstein MD, Gleason RE, Orav J, Haas ST, Friedman AJ, Rein MS, et al. The reproducibility of the revised American Fertility Society classification of endometriosis. Fertil Steril 1993;59:1015–21.

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Associations between serum levels of selected organochlorine compounds and endometriosis in infertile Japanese women ☆

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Abstract

Endocrine-disrupting chemicals (EDCs) have been proposed as risk factors for endometriosis. Persistent organochlorine compounds, a group of suspected EDCs, are present to some extent in almost all human adipose tissue and blood via the food chain. A few animal studies have confirmed that exposure to these compounds can increase the incidence of endometriosis. In this study, we examined the associations between endometriosis and exposure to selected organochlorine compounds, including 8 polychlorinated dibenzo-*p*-dioxins (PCDDs), 10 polychlorinated dibenzofurans (PCDFs), 4 coplanar polychlorinated biphenyls (cPCBs), 36 *ortho*-substituted polychlorinated biphenyls (PCBs), and 13 chlorinated pesticides or their metabolites. The participants were 139 infertile Japanese women who were examined by laparoscopy and diagnosed as either endometriosis cases (Stages II–IV) or controls (Stages 0–I). The serum levels (lipid adjusted) of the targeted organochlorine compounds were in both 58 cases and 81 controls. There were very few differences in the various levels between endometriosis cases and controls. The total serum toxic equivalency (TEQ) value of PCDDs was significantly higher in the controls than in the cases ($P = 0.02$). No other total TEQ values differed between cases and controls. For PCDDs, PCDFs, cPCBs, and PCBs, the multivariate odds ratio was 0.38 [95% confidence interval (CI), 0.12–1.17] and 0.41 (95% CI, 0.14–1.27) for the third and highest quartiles, respectively, compared to the lowest quartile of total TEQ values. A weak, negative dose–response relationship was evident for total TEQs (P for trend of 0.06). The results of this study provide some evidence that serum levels of these organochlorine compounds are not associated with an increased risk of endometriosis in infertile Japanese women.

Keywords: Organochlorines; Polychlorinated dibenzo-*p*-dioxins; Polychlorinated dibenzofurans; Coplanar polychlorinated biphenyls; Endometriosis

1. Introduction

A number of industrial chemicals in the environment mimic (agonists) or antagonize (antagonists) endogenous hormones (Safe, 2000). These substances are referred to as endocrine-disrupting chemicals (EDCs)

☆All participants gave their written informed consent before the laparoscopic examination. The study protocol was approved by the Institutional Review Board of the Jikei University School of Medicine, the National Cancer Center, National Institute for Environmental Studies, and the US Centers for Disease Control and Prevention (CDC).

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because of their ability to interact with the endocrine system. Many of the EDCs are organochlorine compounds, including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), coplanar polychlorinated biphenyls (cPCBs), *ortho*-substituted polychlorinated biphenyls (PCBs), and chlorinated pesticides. PCDDs and PCDFs are formed as byproducts of various industrial chemical reactions and combustion processes, including waste incineration. PCBs and chlorinated pesticides were widely used in industry and agriculture until the 1970s in Japan. Because these compounds are lipid soluble and resistant to metabolism, they tend to bioaccumulate through the food chain. They are also present in human adipose tissue and in the lipid components of blood, mainly because of food intake (Safe, 2000).

There is evidence that EDCs may adversely affect the health of wildlife and humans (Birnbaum and Fenton, 2003; Safe, 2000; Steenland et al., 2004). Some of these chemicals, such as dichlorodiphenyltrichloroethane (DDT) and certain PCBs, are considered to be estrogenic substances (Wolff and Toniolo, 1995), while others, including dioxins, are considered to be anti-estrogenic substances (Krishnan et al., 1995). A recent study revealed that dioxins enact estrogenic effects through estrogen receptor-mediated signaling modulated by the agonist-activated aryl hydrocarbon receptor (AhR) ligand complex (Ohtake et al., 2003). Endogenous estrogens enlarge the endometrial lining of the uterus, and estrogenic substances have been suggested as inducing endometriosis. A study of adult rhesus monkeys revealed that chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in feed (0, 5, or 25 ppt) was associated with a dose-dependent increase in the incidence and severity of endometriosis (Rier et al., 1993). In humans, a few case-control studies have been conducted on the association between serum levels of PCDDs, PCDFs, cPCBs, PCBs and chlorinated pesticides and endometriosis, but the results are inconsistent because of differences in study design, analytical methods, and the organochlorine compounds measured in each study (Boyd et al., 1995; Gerhard and Runnebaum, 1992; Lebel et al., 1998; Mayani et al., 1997; Pauwels et al., 2001). Recently, a cohort study of residents of Seveso, Italy, who were exposed to TCDD from the explosion of a trichlorophenol-manufacturing factory in 1976 revealed a doubled but nonsignificant risk for endometriosis among women with serum TCDD levels of 100 ppt or higher. No clear dose–response relation was found among 19 endometriosis cases, 277 nondiseased women, or 305 uncertain-status cases (Eskenazi et al., 2002). Although in vitro studies using certain organochlorine compounds have suggested many possible endocrine-disrupting mechanisms, such as estrogenic or anti-estrogenic properties, immune suppression, and enzyme induction through binding to the AhR (Nicolopoulou-Stamati and Pitsos, 2001), less is known about the effect of these organochlorine compounds on human endometriosis.

The purpose of this study was to determine the possible association between environmental exposures to selected organochlorine compounds and the prevalence of endometriosis among infertile Japanese women. In a hospital-based case-control study, the serum levels of 8 PCDDs, 10 PCDFs, 4 cPCBs, 36 PCBs, and 13 chlorinated pesticides were measured in laparoscopically confirmed endometriosis patients and in their corresponding references.

2. Materials and methods

2.1. Subjects and sample collection

Eligible women were aged 20–45 years, had complained of infertility, and had consulted doctors in the Department of Obstetrics and Gynecology of the Jikei University School of Medicine in 1999 and 2000. Women who had ever given birth or lactated were not eligible for this study. Sampling was consecutive, and a total of 159 women met these criteria. After we excluded 15 women who did not give their consent and 5 women who did not go through a blood screen or a laparoscopic examination, 139 women were available for analysis. Endometriosis was diagnosed laparoscopically according to the revised classification of the American Fertility Society (1985). Fifty-nine women (42.4%) were assigned to stage 0. Twenty-two (15.8%) were assigned to stage I, 10 (7.2%) to stage II, 23 (16.5%) to stage III, and 25 (18.0%) to stage IV. The 58 women with stage II or higher endometriosis were designated as "cases." Eighty-one women with stage 0 or I were designated as "controls." Among controls, some causal infertility conditions were confirmed laparoscopically: myoma of the uterus (39.5%), polycystic ovary (16.0%), and obstruction of the ovarian duct passage (23.5%). All participants gave their written informed consent before the laparoscopic examination. The study protocol was approved by the Institutional Review Board of the Jikei University School of Medicine, the National Cancer Center, National Institute for Environmental Studies, and the US Centers for Disease Control and Prevention (CDC).

A fasting blood sample was obtained before the laparoscopic examination. Serum was immediately collected by centrifugation, transferred into a stock tube, and stored at −80 °C until analyzed.

2.2. Questionnaire survey

Participants were interviewed face-to-face before the laparoscopic examination by a trained interviewer using

a structured questionnaire. Responses were elicited concerning demographic and anthropometric variables (age, height, and weight), occupation (primary industry, plant worker, office worker, specialist, merchant, housewife, no occupation, and other), education (junior high school, high school, junior college or vocational school, college, graduate school), marital status (married, never married, divorced, widowed, and other), family history of endometriosis, use of alcohol (rare, 1–3 times/month, 1–2 times/week, 3–4 times/week, 5–6 times/week, every day), and smoking habits (never, past, and current). We derived a menstrual history from responses to the following questions: (1) "At what age did you have your first menstrual period?" (2) "In the past year, were your menstrual periods regular? Irregular? Other? Don't know?" (3) "In the past year, what was the usual length of your menstrual cycle?" We also obtained information about potential sources of exposure to endocrine-disrupting compounds (e.g., tampons, oral contraceptives, and pesticides).

2.3. Analytical methods

Serum analyses were performed at the CDC by gas chromatography/high-resolution isotope-dilution mass spectrometry for a total of 71 compounds: 8 PCDDs [2,3,7,8-tetra-chlorodibenzo-*p*-dioxin (CDD), 1,2,3,7,8-pentaCDD, 1,2,3,4,7,8-hexaCDD, 1,2,3,6,7,8-hexaCDD, 1,2,3,7,8,9-hexaCDD, 1,2,3,4,6,7,8-heptaCDD, 1,2,3,4,6,7,9-heptaCDD, 1,2,3,4,6,7,8,9-octaCDD], 10 PCDFs [2,3,7,8-tetra-chlorodibenzofuran (CDF), 1,2,3,7,8-pentaCDF, 2,3,4,7,8-pentaCDF, 1,2,3,4,7,8-hexaCDF, 1,2,3,6,7,8-hexaCDF, 1,2,3,7,8,9-hexaCDF, 2,3,4,6,7,8-hexaCDF, 1,2,3,4,6,7,8-heptaCDF, 1,2,3,4,7,8,9-heptaCDF, 1,2,3,4,6,7,8,9-octaCDF], 4 cPCBs [3,3′,4,4′-tri-chlorobiphenyl (CB), 3,4,4′,5-tetraCB, 3,3′,4,4′,5-pentaCB, 3,3′,4,4′,5,5′-hexaCB], 36 *ortho*-substituted PCBs (International Union of Pure and Applied Chemistry Nos. 18, 28, 44, 49, 52, 66, 74, 87, 99, 101, 105, 110, 118, 128, 138/158, 146, 149, 151, 153, 156, 157, 167, 170, 172, 177, 178, 180, 183, 187, 189, 194, 195, 196/203, 201, 206, 209), and 13 selected persistent chlorinated pesticides or their metabolites [hexachlorobenzene, β-hexachlorocyclohexane, λ-hexachlorocyclohexane, aldrin, heptachlor epoxide, oxychlordane, *trans*-nonachlor, *p,p*′-1,1′-[2,2-dichloroethenylidene]-bis[4-chlorobenzene] (*p,p*′-DDE), dieldrin, endrin, *o,p*′-dichlorodiphenyltrichloroethane (*o,p*′-DDT), *p,p*′-dichlorodiphenyltrichloroethane (*p,p*′-DDT), and mirex]. The analytical methods and quality control procedures have been described previously (DiPietro et al., 1997; Patterson et al., 1987; Turner et al., 1994). Because the PCDDs, PCDFs, cPCBs, PCBs, and chlorinated pesticides are lipophilic and concentrate in the body's lipid stores, including the lipid in serum, the serum levels for these compounds were adjusted for serum lipid levels. Triglycerides and total cholesterol were used in calculating total lipid levels (2.27 × total cholesterol + triglycerides + 62.3) (Phillips et al., 1989). Limits of detection (LODs) on a lipid-adjusted basis were calculated for each sample. Because we could not measure PCB138 and 158, or PCB196 and 203, separately, combined values for PCB138/158 and PCB196/203 were reported. Dioxin toxic equivalency (TEQ) was assessed using a "toxic equivalency factor" (TEF) based upon the relative potency of each congener of the PCDDs, PCDFs, cPCBs, and mono-*ortho*-substituted PCBs compared with 2,3,7,8-TCDD, the most potent of the compounds (Van den Berg et al., 2000). To calculate the TEQ values of PCDDs, PCDFs, cPCBs, and PCBs in this study, the World Health Organization TEF values were assigned to all of the PCDDs, PCDFs, and cPCBs, except for 1,2,3,4,6,7,9-heptaCDD, and PCB105, 118, 156, 157, 167, and 189 (Van den Berg et al., 1998). Because of the small sample volume, the respective serum PCDDs/PCDFs/cPCBs and PCBs could not be measured for each participant. All targeted organochlorine compounds could not be measured for 1 control subject because of the poor conditions under which the serum was maintained.

2.4. Statistical analysis

Characteristics of cases and controls were compared using the χ^2-test and Student's *t*-test. To estimate the value of samples below the LOD for a comparison of serum organochlorine levels between cases and controls, the values < the LOD were assigned one-half of the LOD. Total levels of TCDDs/TCDFs/cPCBs, PCBs, and pesticides were calculated for the sum of the serum molar concentrations of TCDDs/TCDFs/cPCBs, PCBs, and pesticides. The serum organochlorine levels were normalized by log transformation because their distributions were confirmed to be skewed by the Kolmogorov–Smirnoff test. Differences in log-transformed serum organochlorine levels and total TEQs between 2 or more groups were established by Student's *t*-test or an analysis of variance. Odds ratios (ORs), 95% confidence intervals (CIs), and *P* values for trends were obtained by unconditional logistic regression analysis to estimate the association between total TEQ and endometriosis. Cases and controls were divided into 4 groups by the quartile of serum total TEQ values of PCDDs, PCDFs, cPCBs, and PCBs among controls, and crude and adjusted ORs were calculated. Because menstruation was thought to be correlated with the risk for endometriosis (Cramer et al., 1986; Eskenazi and Warner, 1997), ORs for endometriosis were adjusted for menstrual regularity (regular or irregular) and average cycle (days). A *P* value of less than 0.05 (2-tail) was considered statistically significant. All analyses were

conducted using the SAS (Version 8.2) program (SAS Institute Inc., Cary, NC, USA).

3. Results

Mean ages of cases and controls were 32.4 and 32.9 years, respectively (Table 1). Differences in other risk factors for endometriosis between cases and controls were not statistically significant, except for irregularity in the menstrual cycle (11.3% of cases and 32.9% of controls, $P = 0.005$) and the duration of menstrual cycles (28.5 days for cases and 30.4 days in controls, $P = 0.03$).

In this study, 3 dioxin-like chemicals (1,2,3,4,6,7,9-heptaCDD, octaCDF, and 3,3′,4,4′-tetraCB), 4 PCBs (International Union of Pure and Applied Chemistry Nos. 18, 28, 149 and 177), and 2 pesticides (aldrin and endrin) could not be measured because of analytical conditions. Statistical analyses were performed on 7 PCDDs, 9 PCDFs, and 3 cPCBs; 32 PCBs; and 11 pesticides. An analysis of variance showed that the value of log-transformed 1,2,3,6,7,8-hexaCDD was significantly greater in controls than in cases ($P = 0.03$), although the other compounds showed no such significant differences (data not shown).

We analyzed the association between total TEQ values and endometriosis (Table 2). The serum total TEQ value of PCDDs was significantly higher in controls than in cases ($P = 0.02$). No other total TEQ values differed between cases and controls. Furthermore, we compared total levels of PCDDs, PCDFs,

Table 1
Baseline characteristics of women with and without endometriosis

Characteristic	Cases ($n = 58$)		Controls ($n = 81$)		P value
	No.[a]	(%)	No.[a]	(%)	
Age, mean (SD), yr	32.4	(3.4)	32.9	(3.9)	0.43[b]
Height, mean (SD), cm	159.0	(5.6)	158.1	(5.4)	0.34[b]
Weight, mean (SD), kg	51.1	(5.6)	52.5	(8.9)	0.33[b]
Education					
High school or less	9	(17.0)	13	(16.9)	
Junior college or vocational school	30	(56.6)	31	(40.3)	
College or graduate school	14	(26.4)	33	(42.9)	0.13[c]
Family history of endometriosis					
Yes	1	(2.0)	5	(6.6)	
No	49	(98.0)	71	(93.4)	0.24[c]
Age menses began, (SD), yr	12.5	(1.3)	12.4	(1.6)	0.66[b]
Oral contraceptive use					
Used	5	(9.4)	12	(15.6)	
Never used	48	(90.6)	65	(84.4)	0.31[c]
Regularity of menstrual cycle					
Regular	47	(88.7)	51	(67.1)	
Irregular	6	(11.3)	25	(32.9)	0.005[c]
Duration of menstrual cycles, mean (SD), days	28.5	(3.2)	30.4	(5.5)	0.03[b]
Tampon use					
Used	41	(77.4)	62	(80.5)	
Never used	12	(22.6)	15	(19.5)	0.66[c]
Smoking status					
Never	36	(69.2)	50	(64.9)	
Former	9	(17.3)	11	(14.3)	
Current	7	(13.5)	16	(20.8)	0.55[c]
Alcohol consumption					
Daily	10	(18.9)	15	(19.5)	
Weekly	22	(41.5)	24	(31.2)	
Occasionally/none	21	(39.6)	38	(49.4)	0.45[c]

Abbreviation: SD, standard deviation.
[a]Number of participants for each item varied because of missing information.
[b]Student's t-test.
[c]Chi-square test.

Table 2
Serum total TEQ values of PCDDs, PCDFs, cPCBs, and PCBs among endometriosis cases and controls

Analyte	No.	TEQ values (pg TEQ/g lipid)				P value for difference
	Total (cases/controls)	Cases		Controls		
		Median	(Q_1, Q_3)	Median	(Q_1, Q_3)	
PCDDs	57/80	7.43	(5.17, 9.47)	8.63	(6.39, 10.75)	0.02
PCDFs	57/80	7.80	(6.07, 9.06)	7.51	(6.32, 9.00)	0.64
cPCBs	57/80	4.61	(3.41, 5.92)	5.14	(3.46, 7.41)	1.00
PCBs	57/80	3.40	(2.59, 4.22)	3.59	(2.37, 5.03)	0.79
PCDDs/PCDFs/cPCBs	57/80	19.40	(16.08, 25.26)	21.58	(17.40, 26.90)	0.23
Sum	56/80	22.76	(19.73, 29.14)	25.07	(20.27, 31.84)	0.23

Q_1 = 25th percentile; Q_3 = 75th percentile.

Table 3
Risk for endometriosis according to quartile of serum total TEQ values of PCDDs, PCDFs, cPCBs, and PCBs

Variable	Total no.	Serum total TEQ values of PCDDs, PCDFs, cPCBs and PCBs (pg TEQ/g lipid)				P value for trend
		Quartile 1 ≦20.27	Quartile 2 >20.27–25.07	Quartile 3 >25.07–31.84	Quartile 4 >31.84	
No. of cases	56	17	17	12	10	
No. of controls	80	20	20	20	20	
Crude OR (95% CI)	56/80	1.00 (reference)	1.00 (0.40, 2.50)	0.71 (0.27, 1.85)	0.59 (0.22, 1.59)	0.23
No. of cases	51[b]	17	16	10	8	
No. of controls	70[b]	17	16	18	19	
Adjusted OR (95% CI)[a]	52/70	1.00 (reference)	0.97 (0.36, 2.63)	0.38 (0.12, 1.17)	0.41 (0.14, 1.27)	0.06

[a]OR was adjusted for menstrual regularity (regular or irregular) and average cycle length (days).
[b]Number of total participants varied because of missing information.

cPCBs, PCBs, and pesticides between the 2 groups. The serum level of PCDDs was significantly higher in controls than in cases ($P = 0.04$). No statistically significant differences were found in the total levels of PCDFs, cPCBs, PCBs, pesticides, and the sum of organochlorines between groups (data not shown).

We found nonsignificantly lower crude ORs for women in the third or fourth quartile of total TEQ compared to women in the first quartile (OR = 0.71, 95% CI, 0.27–1.85, and OR = 0.59, 95% CI, 0.22–1.59, respectively) (Table 3). Similarly, adjusted ORs for women in the third or fourth quartile of total TEQs were lower than those for the first, but not significantly so (OR = 0.38, 95% CI, 0.12–1.17, and OR = 0.41, 95% CI, 0.14–1.27, respectively). A weak inverse dose–response relationship was evident for total TEQs (P for trend of 0.06). We also analyzed the ORs by quartile of total serum levels of PCDDs/PCDFs/cPCBs, PCBs, and pesticides but found no significantly increased or decreased ORs. The serum organochlorine levels in women in whom stages I, II, III, and IV were diagnosed by laparoscopy did not differ significantly from the levels in women with stage 0 (data not shown).

4. Discussion

Our study found no evidence of an increased risk for endometriosis related to serum levels of several organochlorine compounds among infertile Japanese women. Furthermore, the serum level of 1,2,3,6,7,8-hexaCDD was significantly lower in endometriosis patients than in controls. This result might be attributed to multiple comparisons because 62 compounds were simultaneously analyzed in this study. However, the total TEQ values of PCDDs, PCDFs, cPCBs, and PCBs were lower in cases than in controls, and the endometriosis risk for women with higher total TEQ values tended to be lower than for those with lower total TEQ values, although not significantly so. These results were not consistent neither with a previous animal study of Rier et al. (1993) nor with the cohort study in Seveso (Eskenazi et al., 2002), possibly because of the difference in exposure levels. Estimated TCDD exposure levels among Seveso residents were similar to those in monkeys in the 25-ppt group in an earlier study by Rier et al. (Bois and Eskenazi, 1994). The median serum TCDD levels reported in Seveso for the women with

endometriosis were 77.3 vs. 61.0 ppt for the controls, whereas the corresponding median total TEQ levels among our study participants were 17.8 ppt in cases vs. 19.2 ppt in controls. According to the animal studies of Foster et al. (1997) and Yang et al. (2000), high-dose exposure to TCDD increases the size of an endometrial fragment autotransplanted to the abdominal or pelvic cavity; in contrast, low-dose exposure decreases implant size. Thus, high TCDD exposure might stimulate endometrial tissue, but low exposure levels appear to be inhibitory, perhaps because of the anti-estrogenic mechanism of TCDD (Scialli, 2001). Pauwels et al. (2001) investigated the association between dioxin-like compounds and endometriosis in a human epidemiological study and reported no statistically significant associations between dioxin-like compounds and the occurrence of endometriosis. As in the study of Pauwels et al. (2001), we analyzed the risk of endometriosis by subdividing serum TEQ values into 4 categories (<20.0 pg TEQ/g, 20.1–60.0 pg TEQ/g, 60.1–100 pg TEQ/g, and >100 pg TEQ/g). Crude ORs of the groups with 20.1–60.0 pg TEQ/g, 60.1–100 pg TEQ/g, and >100 pg TEQ/g were 0.93 (95% CI, 0.29–3.30), 0.53 (95% CI, 0.14–1.94), and 3.6 (95% CI, 0.37–34.94) compared to the group with <20.0 pg TEQ/g. The risks of endometriosis were decreased with low TEQ values, but the risk of endometriosis was increased with high TEQ values, in the study of Pauwels et al. The total TEQ values in our study ranged from 1.38 to 44.7 TEQ/g; thus, our results were consistent with the results of Pauwels et al.

Our study has several strengths. First, we applied a clinical best definition of cases and controls. Endometriosis has been defined as the presence of endometrial glands and stroma outside of the uterine cavity, and the presence of an ectopic endometrium can be accurately determined only by inspection of the pelvic cavity during laparoscopy (Holt and Weiss, 2000; Zondervan et al., 2002). In the present study, cases and controls were diagnosed by laparoscopic examination. Moreover, we conducted consecutive sampling, so potential selection bias was excluded. Secondly, the detailed interviews were performed by the same trained interviewer before the laparoscopic examination. This interviewer validated each questionnaire so that misclassification of this information might be minimized. Because no participants knew whether they had endometriosis before the examination, recall bias can be ruled out. Detailed questionnaires were designed to detect possible confounding factors for the risk of endometriosis such as the regularity and duration of menstrual cycles. Adjustments for such variables were performed when calculating ORs for endometriosis in relation to the total TEQ values of serum levels of dioxin-like chemicals. Although in our study differences were not significant among age, smoking, and age at menarche between cases and controls, age and age at menarche have been reported to be risk factors for endometriosis, and smoking has been reported to be protective for endometriosis (Cramer et al., 1986; Eskenazi and Warner, 1997). Thus, we adjusted these factors in calculating the ORs for endometriosis in relation to the total TEQ values, but again no significant ORs were found.

On the other hand, our study has some limitations. Many participants showed values <LOD on some organochlorine compounds because of the low serum levels or small sample volume. Therefore, we evaluated the risk for total TEQ values. To assess the effects of dioxin mixtures, dioxin toxicity was assessed using a TEF that estimated the toxic potency of a compound relative to TCDD. The TEF values were consensus estimates based on an evaluation of all of the data (Van den Berg et al., 1998). In our study, the median total TEQ value of 19 PCDDs, PCDFs, cPCBs, and 6 PCBs was 25.07 pg TEQ/g lipid in the controls. This value is slightly higher than those previously reported for other Japanese populations by Arisawa et al. (2003). They measured 7 PCDDs, 10 PCDFs, and 12 PCBs among randomly selected groups of 131 men and 122 women who had no occupational exposure to dioxins, and the median total TEQs in men and women were 17 and 16 pg TEQ/g lipid, respectively. However, these values may be underestimates because the values <LOD were assigned to zero. When values <LOD were assigned to zero in our study, the median total TEQ serum levels of PCDDs, PCDFs, cPCBs, and PCBs were computed as 19.2 pg TEQ/g lipid in the controls, a value roughly consistent with that reported by Arisawa et al. (2003). We calculated the median total TEQ values of PCDDs and PCDFs to be 12.5 pg TEQ/g lipid in our controls, while the median total TEQ values of PCDDs and PCDFs previously reported were 9.8–22.9 pg TEQ/g lipid in Japan (Arisawa et al., 2003; Kumagai et al., 2000), 16.1–40.8 pg TEQ/g lipid in Germany (Papke, 1998; Wittsiepe et al., 2000), and 14.6–20.6 pg TEQ/g lipid in Canada (Ayotte et al., 1997; Ryan et al., 1997). The TEF exposure metric, which is based on Ah receptor induction, may not be appropriate for the estrogenic or anti-estrogenic activity of the chemicals measured. Some of the chemicals have been shown to have primarily anti-estrogenic characteristics (TCDD), while others have been shown to be estrogenic (PCBs, along with some of the organochlorine pesticides). Some of the DDT congeners are also anti-androgenic. It is difficult to propose an alternative metric that is more appropriate at this time, as no well-validated metrics have been developed for total estrogenic activity, anti-androgenic activity, and anti-estrogenic activity. However, it might be worthwhile to attempt to analyze these data using some alternative metrics. Another study limitation involves endogenous estrogen exposure.

Because the growth of endometrial cells depends on estrogen, the endogenous estrogen level is an important risk factor for endometriosis. Endogenous estrogen levels were not measured in the present study. Other related factors, such as menstrual regularity and average cycle, were adjusted in calculating the endometriosis risk from organochlorine exposure. Furthermore, participants of the present study had complained of infertility, and some of the controls had other estrogen-related diseases, such as myoma of the uterus (39.5%) and polycystic ovary (16.0%), according to the laparoscopic findings. Thus, the present controls may not reflect women in general. Finally, in our case-control study, endometriosis could have affected the serum levels of these organochlorine compounds because regularity and short menstrual cycles might increase the total menstrual flow, thus decreasing the women's serum levels.

Human exposure to PCDDs, PCDFs, and cPCBs occurs almost exclusively through food consumption, particularly of fish and meat. Many kinds of fish from several supermarkets in Japan reportedly contain elevated levels of PCDDs, PCDFs, and cPCBs, and the mean daily intake of these chemicals from the ingestion of fish and shellfish was higher than that of other foods (Tsutsumi et al., 2001). These findings suggest that people who eat fish often should have a higher level of PCDDs, PCDFs, and cPCBs in their system. Some studies have investigated the associations between the serum level of organochlorine compounds and the frequency of fish intake in Japan, and most of these results have shown that fish consumption is associated positively with serum levels of organochlorines (Arisawa et al., 2003; Kitamura et al., 2000; Kumagai et al., 2000). Among women in the present study, we analyzed the association between serum total TEQ values of PCDDs, PCDFs, cPCBs, and PCBs and 6 items of food intake, and we also found that higher total TEQ values of PCDDs, PCDFs, cPCBs, and PCBs correlate with an increased frequency of fish intake (unpublished data).

In summary, serum levels of these targeted organochlorine compounds did not differ between endometrial cases and controls except for 1,2,3,6,7,8-hexaCDD. The risk for endometriosis among participants with higher total TEQ values of PCDDs, PCDFs, cPCBs, and PCBs was nonsignificantly lower than that for participants with lower total TEQ values. These findings suggest that higher serum levels of these organochlorine compounds are not associated with an increased risk for endometriosis among infertile Japanese women.

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References

American Fertility Society, 1985. Revised American Fertility Society Classification of Endometriosis. Fertil. Steril. 43, 351–352.

Arisawa, K., Matsumura, T., Tohyama, C., Saito, H., Satoh, H., Nagai, M., Morita, M., Suzuki, T., 2003. Fish intake, plasma omega-3 polyunsaturated fatty acids, and polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzo-furans and co-planar polychlorinated biphenyls in the blood of the Japanese population. Int. Arch. Occup. Environ. Health 76, 205–215.

Ayotte, P., Dewailly, E., Ryan, J.J., Bruneau, S., Lebel, G., 1997. PCBs and dioxin-like compounds in plasma of adult Inuit living in Nunavik (Arctic Quebec). Chemosphere 34, 1459–1468.

Birnbaum, L.S., Fenton, S.E., 2003. Cancer and developmental exposure to endocrine disruptors. Environ. Health Perspect. 111, 389–394.

Bois, F.Y., Eskenazi, B., 1994. Possible risk of endometriosis for Seveso, Italy, residents: an assessment of exposure to dioxin. Environ. Health Perspect. 102, 476–477.

Boyd, J., Clark, G., Walmer, D., Patterson, D., Needham, L., Lucier, G., 1995. Endometriosis and the environment: biomarkers of toxin exposure. Endometriosis 2000 (Bethesda, MD).

Cramer, D.W., Wilson, E., Stillman, R.J., Berger, M.J., Belisle, S., Schiff, I., Albrecht, B., Gibson, M., Stadel, B.V., Schoenbaum, S.C., 1986. The relation of endometriosis to menstrual characteristics, smoking, and exercise. J. Am. Med. Assoc. 255, 1904–1908.

DiPietro, E.S., Lapenza Jr., C.R., Cash, T.P., Turner, W.E., Green, V.E., Gill, J.B., 1997. A fast universal automated cleanup system for the isotope-dilution high resolution mass spectrometric analysis of PCDDs, PCDFs, coplanar-PCBs, PCB congeners, and persistent pesticides from the same serum sample. Organohalogen Compd. 31, 26–31.

Eskenazi, B., Warner, M.L., 1997. Epidemiology of endometriosis. Obstet. Gynecol. Clin. N. Am. 24, 235–258.

Eskenazi, B., Mocarelli, P., Warner, M., Samuels, S., Vercellini, P., Olive, D., Needham, L.L., Patterson Jr., D.G., Brambilla, P., Gavoni, N., Casalini, S., Panazza, S., Turner, W., Gerthoux, P.M., 2002. Serum dioxin concentrations and endometriosis: a cohort study in Seveso, Italy. Environ. Health Perspect. 110, 629–634.

Foster, W.G., Ruka, M.P., Gareau, P., Foster, R.A., Janzen, E.G., Yang, J.Z., 1997. Morphologic characteristics of endometriosis in the mouse model: application to toxicology. Can. J. Physiol. Pharmacol. 75, 1188–1196.

Gerhard, I., Runnebaum, B., 1992. The limits of hormone substitution in pollutant exposure and fertility disorders. Zentralbl Gynakol 114, 593–602 (in German).

Holt, V.L., Weiss, N.S., 2000. Recommendations for the design of epidemiologic studies of endometriosis. Epidemiology 11, 654–659.

Kitamura, K., Kikuchi, Y., Watanabe, S., Waechter, G., Sakurai, H., Takada, T., 2000. Health effects of chronic exposure to polychlorinated dibenzo-*p*-dioxins (PCDD), dibenzofurans (PCDF) and coplanar PCB (Co-PCB) of municipal waste incinerator workers. J. Epidemiol. 10, 262–270.

Krishnan, V., Porter, W., Santostefano, M., Wang, X., Safe, S., 1995. Molecular mechanism of inhibition of estrogen-induced cathepsin D gene expression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in MCF-7 cells. Mol. Cell. Biol. 15, 6710–6719.

Kumagai, S., Koda, S., Miyakita, T., Yamaguchi, H., Katagi, K., Yasuda, N., 2000. Polychlorinated dibenzo-*p*-dioxin and dibenzofuran concentrations in the serum samples of workers at continuously burning municipal waste incinerators in Japan. Occup. Environ. Med. 57, 204–210.

Lebel, G., Dodin, S., Ayotte, P., Marcoux, S., Ferron, L.A., Dewailly, E., 1998. Organochlorine exposure and the risk of endometriosis. Fertil. Steril. 69, 221–228.

Mayani, A., Barel, S., Soback, S., Almagor, M., 1997. Dioxin concentrations in women with endometriosis. Hum. Reprod. 12, 373–375.

Nicolopoulou-Stamati, P., Pitsos, M.A., 2001. The impact of endocrine disrupters on the female reproductive system. Hum. Reprod. Update 7, 323–330.

Ohtake, F., Takeyama, K., Matsumoto, T., Kitagawa, H., Yamamoto, Y., Nohara, K., Tohyama, C., Krust, A., Mimura, J., Chambon, P., Yanagisawa, J., Fujii-Kuriyama, Y., Kato, S., 2003. Modulation of oestrogen receptor signalling by association with the activated dioxin receptor. Nature 423, 545–550.

Papke, O., 1998. PCDD/PCDF: human background data for Germany, a 10-year experience. Environ. Health Perspect. 106, 723–731.

Patterson Jr., D.G., Hampton, L., Lapeza Jr., C.R., Belser, W.T., Green, V., Alexander, L., Needham, L.L., 1987. High-resolution gas chromatographic/high-resolution mass spectrometric analysis of human serum on a whole-weight and lipid basis for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Anal. Chem. 59, 2000–2005.

Pauwels, A., Schepens, P.J., D'Hooghe, T., Delbeke, L., Dhont, M., Brouwer, A., Weyler, J., 2001. The risk of endometriosis and exposure to dioxins and polychlorinated biphenyls: a case-control study of infertile women. Hum. Reprod. 16, 2050–2055.

Phillips, D.L., Pirkle, J.L., Burse, V.W., Bernert Jr., J.T., Henderson, L.O., Needham, L.L., 1989. Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. Arch. Environ. Contam. Toxicol. 18, 495–500.

Rier, S.E., Martin, D.C., Bowman, R.E., Dmowski, W.P., Becker, J.L., 1993. Endometriosis in rhesus monkeys (*Macaca mulatta*) following chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Fundam. Appl. Toxicol. 21, 433–441.

Ryan, J.J., Dewailly, E., Gilman, A., Laliberte, C., Ayotte, P., Rodrigue, J., 1997. Dioxin-like compounds in fishing people from the Lower North Shore of the St. Lawrence River, Quebec, Canada. Arch. Environ. Health 52, 309–316.

Safe, S.H., 2000. Endocrine disruptors and human health—is there a problem? An update. Environ. Health Perspect. 108, 487–493.

Scialli, A.R., 2001. Tampons, dioxins, and endometriosis. Reprod. Toxicol. 15, 231–238.

Steenland, K., Bertazzi, P., Baccarelli, A., Kogevinas, M., 2004. Dioxin revisited: developments since the 1997 IARC classification of dioxin as a human carcinogen. Environ. Health Perspect. 112, 1265–1268.

Tsutsumi, T., Yanagi, T., Nakamura, M., Kono, Y., Uchibe, H., Iida, T., Hori, T., Nakagawa, R., Tobiishi, K., Matsuda, R., Sasaki, K., Toyoda, M., 2001. Update of daily intake of PCDDs, PCDFs, and dioxin-like PCBs from food in Japan. Chemosphere 45, 1129–1137.

Turner, W.E., DiPietro, E.S., Cash, T.P., McClure, P.C., Patterson, D.G.J., Shirkhan, H., 1994. An improved SPE extraction and automated sample cleanup method for serum PCDDs, PCDFs, and coplanar PCBs. Organohalogen Compd. 19, 31–35.

Van den Berg, M., Birnbaum, L., Bosveld, A.T., Brunstrom, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., Kubiak, T., Larsen, J.C., van Leeuwen, F.X., Liem, A.K., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F., Zacharewski, T., 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ. Health Perspect. 106, 775–792.

Van den Berg, M., Peterson, R.E., Schrenk, D., 2000. Human risk assessment and TEFs. Food Addit. Contam. 17, 347–358.

Wittsiepe, J., Schrey, P., Ewers, U., Wilhelm, M., Selenka, F., 2000. Decrease of PCDD/F levels in human blood—trend analysis for the German population, 1991–1996. Environ. Res. 83, 46–53.

Wolff, M.S., Toniolo, P.G., 1995. Environmental organochlorine exposure as a potential etiologic factor in breast cancer. Environ. Health Perspect. 103 (Suppl. 7), 141–145.

Yang, J.Z., Agarwal, S.K., Foster, W.G., 2000. Subchronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin modulates the pathophysiology of endometriosis in the cynomolgus monkey. Toxicol. Sci. 56, 374–381.

Zondervan, K.T., Cardon, L.R., Kennedy, S.H., 2002. What makes a good case-control study? Design issues for complex traits such as endometriosis. Hum. Reprod. 17, 1415–1423.

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ORIGINAL PAPER

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Human glutathione S-transferase A1, T1, M1, and P1 polymorphisms and susceptibility to prostate cancer in the Japanese population

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Abstract *Purpose*: The incidence of prostate cancer is increasing in low-risk populations such as Japanese. One of the causes of this increase is considered to be associated with the Western diet, especially the high intake of red meat and fat. Glutathione S-transferase (GST) A1, T1, M1, and P1 are phase II enzymes that are important for activation and detoxification of chemical carcinogens. *Methods*: In this study, 190 Japanese male patients with prostate cancer and 294 healthy controls, frequency-matched for age, were compared for frequencies of *GSTA1*, *GSTT1*, *GSTM1*, and *GSTP1* genotypes. *Results*: Among smokers, the frequency of the *GSTA1*A/*B* or **B/*B* genotype in patients with prostate cancer (27.8%) showed a statistically significant increase compared with the control group frequency (18.2%; odds ratio [OR] = 1.72; 95% CI, 1.01–2.94). In addition, the frequency of *GSTT1* nondeletion genotype was associated with prostate cancer among smokers (OR = 1.68; 95% CI, 1.06–2.68). The OR of carrying the combined genotyping of *GSTA1*A/*B* or **B/*B* and *GSTT1* nondeletion was 2.08 (95% CI, 1.14–3.80) with the combined genotyping of *GSTA1*A/*A* and *GSTT1* null as a reference. On the other hand, no significant associations were observed for genotypes of *GSTM1* and *GSTP1 I105V*. *Conclusions*: These findings suggest that the *GSTA1* and *GSTT1* polymorphisms are associated with prostate cancer susceptibility, especially among smokers.

Keywords *GSTA1* · *GSTT1* · Polymorphism · Prostate cancer

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Introduction

Prostate cancer is the most common cancer in men in Europe, North America, and some parts of Africa. The incidence varies widely between ethnic populations and countries. The lowest rates are usually in Asia, and the highest are in North America and Scandinavia, especially in African-American people in the USA.

Migration studies have shown that when Japanese people move from Japan to the USA, the incidence of prostate cancer in these people increases; however, the increase is only to about 50% of the rate for Caucasian people and to 25% of that for African-American people in the USA (Gronberg 2003). These findings suggest that these differences are caused not only by exposure to external risk factors but also by a combination of underlying differences such as genetic susceptibility.

Several studies (Hayes et al. 1999, Veierod et al. 1997) suggest that prostate cancer is associated with a western lifestyle and, in particular, a diet that includes a high intake of red meat, which results in the formation of very potent carcinogens such as heterocyclic amines. For example, PhIP can induce a large increase in mutant frequencies in the rat prostate (Stuart et al. 2000), and ^{32}P-postlabeling analysis of DNA demonstrated that PhIP-DNA adducts are produced in all lobes of the prostates of rats receiving PhIP (Shirai et al. 1997).

Most environmental carcinogens are metabolized via complex enzymatic mechanisms involving activation and inactivation. There are genetic differences in some of these metabolisms, and these individual variations may modulate cancer risk. The glutathione S-transferases (GSTs) are a large family of phase II enzymes that facilitate the detoxification of various carcinogens, therapeutic drugs, environmental toxins, and products of oxidative stress.

GSTA1, an alpha class enzyme, is the most abundant GST found in the liver. It catalyzes the reduction of the carcinogenic *N*-acetoxy derivative back to the parent amine (Lin et al. 1994). Recently, a polymorphism of