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.

Tsuchiya. AhRR polymorphism and endometriosis. Fertil Steril 2005.