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Androgen receptor cytosine, adenine, and guanine trinucleotide repeat polymorphism in Korean patients with endometriosis: A case-control study

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ABSTRACT

Study objective: : To investigate the association between the androgen receptor (AR) cytosine, adenine, and guanine (CAG) repeat polymorphisms and endometriosis.
Study design: : A prospective case-control, genetic association study was performed on women with surgically proven endometriosis (n = 421) and controls free of endometriosis (n = 349). AR CAG repeat lengths were determined from peripheral blood samples. The difference in the frequency of each alleles were compared in patients with endometriosis and controls using Chi-square test.
Main results: : No significant difference in biallelic length mean between patients and controls was observed. Alleles containing 24 CAG repeats were significantly more frequent in stage I–II (mild) endometriosis than in the control samples (19.8% and 13.3%, respectively; OR 1.60, 95% CI 1.04-2.47). Additionally, a higher frequency of both alleles with 24 or more CAG repeats was observed in individuals with mild endometriosis, in comparison with the controls (25.6% and 15.2%, respectively; OR 1.92, 95% CI 1.09-3.38).
Conclusions: AR gene CAG repeat polymorphisms are associated with the increased risk of mild endometriosis.

Introduction

Endometriosis is a benign yet progressive and recurrent disease that is defined by the presence of endometrial tissue outside the uterine cavity. It may present with either minimally with classic spots of endometriosis without any anatomical distortion or moderate-to-severe cases with ovarian endometriomas and extensive adhesions within the pelvic cavity. In the normal endometrium, cyclic endometrial proliferation and differentiation depend on androgen signaling [1–3]. Androgen receptors (ARs) are expressed in the lesion of women with stage III or IV endometriosis, and exogenous androgen administration induced abnormal regulation of endometrial repair in a murine model [4,5]. Thus, AR may play a role in the development of endometriosis, most likely by disrupting endometrial tissue repair in ectopic locations.

The gene encoding AR is located on the X chromosome and contains a highly polymorphic cytosine, adenine, and guanine (CAG) trinucleotide repeat sequence in the 5′ terminal region of exon 1. Functional studies showed an inverse correlation between the length of this repeat and AR activity—shorter CAG repeats were associated with a higher receptor sensitivity to androgen, and vice versa [6]. CAG repeat polymorphism, leading to aberrant androgen sensitivity was found to increase the risk of several diseases.

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Longer CAG repeats were associated with impaired sperm production, male infertility, and spinobulbar muscular atrophy [7,8], while shorter repeats were associated with the increased risk of benign prostatic hyperplasia [9], prostate cancer in men [10,11], and PCOS in women [12].

A number of studies have examined the relationship between CAG repeat length and the risk of developing endometriosis, but the results have been inconclusive. An increase in the risk of endometriosis was observed for patients with 19 and 21 CAG repeats in two studies [13,14]. However, a study investigating endometriosis risk in Italian women concluded that the AR CAG repeat length was not an important factor for the genetic predisposition to endometriosis [15].

The aim of this study was to investigate the association between the endometriosis of variable severity and AR gene CAG repeat polymorphism.

Material and methods

Subjects

Peripheral blood was obtained from a total of 770 subjects between March 2003 and October 2006 at Seoul National University Hospital, Seoul, Korea. All subjects were native Korean. A total of 421 patients had surgical and histologic evidence of endometriosis, whereas 349 patients with no surgical or pathological evidence of endometriosis served as controls. The indications for surgery or diagnostic laparoscopy among the endometriosis group included dysmenorrhea, pelvic pain, infertility and adnexal mass. The indications for surgery or diagnostic laparoscopy among the control group and patients with stage-I/II endometriosis were infertility, benign ovarian cyst, pelvic pain or dysmenorrhea, tubal ligation, carcinoma in situ of the uterine cervix, ectopic pregnancy and the need for tubal reanastomosis. Patients with uterine leiomyoma, adenomyosis, or gynecologic malignancies were excluded from both cases and controls. The severity of endometriosis was assessed using the revised American Society for Reproductive Medicine classification [16], and subjects were classified either as having mild (stage-I/II) or moderate-to-severe (stage-III/IV) disease. The presence of ovarian endometrioma was recorded separately as either unilateral or bilateral for further analysis. None of the participants received hormone therapy within 12 months prior to our study. This study was approved by the Institutional Review Board at Seoul National University Hospital, and written informed consents were obtained from all enrolled subjects (IRB No. 0202-088-003).

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using a commercial kit (Wizard DNA Purification Kit, Promega, Madison, WI, USA), following standard protocols. The CAG repeat region of the AR gene was amplified with polymerase chain reaction (PCR) using forward (5′-TGC GCG AAG TGA TCC AGA AC-3′) and reverse (5′-CTT GGG GAG AAC CAT CCT CA-3′) primers as described previously [13]. PCR was conducted in a total volume of 25 μL containing 0.05 μg of genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTPs (deoxynucleotide triphosphates), 0.08 nM of each primer, and 0.625 U *Taq* polymerase (Takara, Shiga, Japan). The following conditions were used for the fluorescent PCR: initial denaturation, 5 min at 94 °C; 35 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min; and a final extension for 10 min at 72 °C. The size of the PCR product was determined using GeneScan 3.7.1 software (Applied Biosystems, Foster City, CA, USA). The CAG repeat length was calculated for each allele in the genotypic pair.

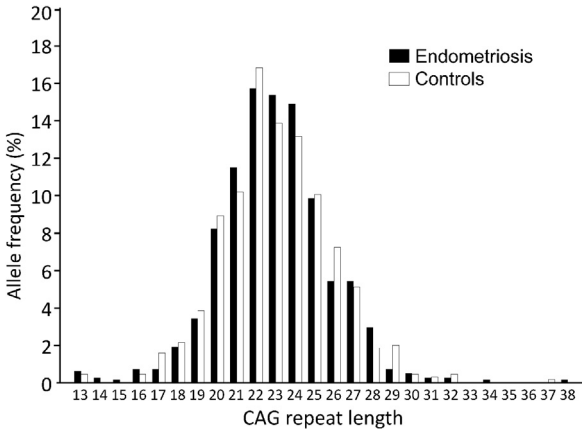

Fig. 1. Distribution of androgen receptor (AR) cytosine, adeninie, and guanine (CAG) repeat length in patients with endometriosis and controls.

Statistical analysis

Allele frequency distribution was calculated. Subgroup analyses were performed according to the severity of the disease and the status of ovarian endometrioma. The association between these variables and the subgroups was evaluated using the unpaired *t*-test, while the odds ratios (ORs) and 95% confidence intervals (CIs) were used to compare categorical variables. All statistical analyses were performed using Statistical Package for the Social Science (SPSS) software (version 22.0, SPSS Inc., Chicago, IL, USA).

Results

All attempted genotyping was successful without missing data. Twenty-three different CAG repeat alleles (13–38 repeats) were found in the subjects enrolled in our study. The distributions of allele frequencies in patients with endometriosis and controls are presented in Fig. 1. In both patients with endometriosis and controls, the most frequent allele contained 22 repeats (15.9% and 17.0% in patients and controls, respectively), followed by allele with 23 repeats (15.6% and 14.0% in patients and controls, respectively).

The 24 CAG repeat alleles were significantly more frequent in stage-I/II endometriosis compared to controls (19.8% and 13.3%, respectively; odds ratio [OR] 1.60, 95% CI 1.04-2.47, Fig. 2). The

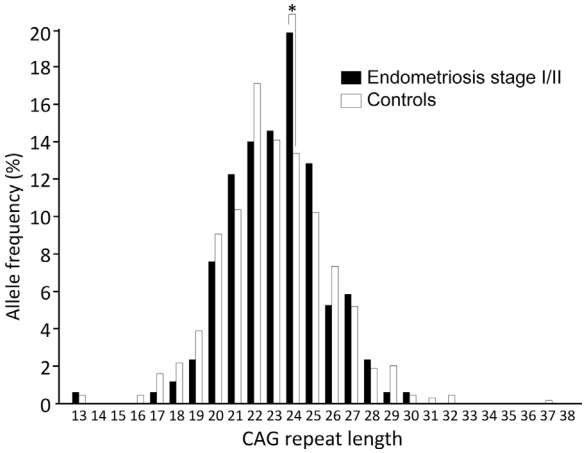

Fig. 2. Distribution of androgen receptor (AR) cytosine, adeninie, and guanine (CAG) repeat length in the patients with stage-I/II endometriosis and controls.