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ARTICLE

CYP17 and CYP2C19 gene polymorphisms in patients with endometriosis

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Abstract Endometriosis seems to be the result of a complex interaction between environmental factors and various genes. In this regard, the cytochrome subfamily 17 (CYP17) may play an important role by altering the biosynthesis of sex steroids. CYP2C19 is also an important member of the cytochrome P450 (CYP) family, and related mutations may result in an inability to fully metabolize environmental chemicals and cytokines, leading to several diseases. This study sought to determine whether there is a relationship between endometriosis and CYP17 T>C, CYP2C19*2 and CYP2C19*3 polymorphisms. When samples from 46 patients with endometriosis and 39 healthy controls were analysed, A2A2 type mutation of the CYP17 gene was observed to be more frequent in patients with endometriosis (34.8 versus 7.7%, $P = 0.003$). No association was found between the severity of endometriosis and CYP2C19*2 or CYP2C19*3 polymorphisms of the CYP2C19 gene. These results suggest that mutations related with sex steroid metabolism seem to have an important role in endometriosis. However, the relation between detoxification ability and endometriosis should be examined in further studies with larger sample sizes.

KEYWORDS: CYP2C19, CYP17, cytochrome P450, endometriosis, gene polymorphism

Introduction

The classic definition of endometriosis is the presence of endometrial gland and stoma outside the uterus (Kitawaki et al., 2002). The exact aetiology is not clear, but implantation after the reflux of endometrial cells through the Fallopian tubes to the peritoneum is accepted as the primary theory (Kitawaki et al., 2001), according to which, every woman of reproductive age having menstrual bleeding should be a candidate for endometriosis. However,

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although it may depend on the examined cohort of patients, only 5—10% of women of childbearing age are estimated to have endometriosis (Kitawaki et al., 2002).

In the available literature, genetic-related problems as well as hormonal influences and immunological alterations are thought to be associated with disease susceptibility in cases suffering from endometriosis (Bulun et al., 1999, 2002, 2004). Although the data are inconclusive, endometriosis seems to be the result of a complex interaction between environmental factors and various genes rather than being monogenetic in origin (De Carvalho et al., 2007; Mitrunen and Hirvonen, 2003).

A series of polymorphisms and mutations related to the cytochrome P450 (CYP) enzyme complex is thought to play a role in the pathogenesis of endometriosis (Falconer et al., 2007). The subfamily 17 (CYP17) has a main role in the biosynthesis of sex steroids (Kado et al., 2002; Vietri et al., 2008). The CYP17 gene encodes the cytochrome P450c17α (Picado-Leonard and Miller, 1987) and mediates both 17α-hydroxylase and 17,20-lyase, which have a role in androgen metabolism. The presence of a single nucleotide polymorphism (T>C) in the 5′-promoter region of the CYP17 creates a new recognition site for the restriction enzyme *MspA*1 and has been used to designate two alleles, A1 and A2 (Haiman et al., 1999). The variant of this polymorphism (A2) has been postulated to alter the sex steroid cycle, resulting in an increased concentration of oestrogens (Feigelson et al., 1998; Haiman et al., 1999).

CYP2C19 is also an important member of the CYP family, and encodes enzymes that are shown to be important in the metabolism of many drugs (Yadav et al., 2008). In addition, CYP2C19 is also known to be involved in the detoxification of potential carcinogens (Kappers et al., 2001) or the bioactivation of some environmental procarcinogens (Fujita and Kamataki, 2001a,b; Yamazaki et al., 2004). Two known allelic variants of cytochrome P450 2C19 are CYP2C19*2 (G682A) and CYP2C19*3 (G636A), which differ from the wild-type CYP2C19*1 by a single nucleotide substitution. Goldstein and de Morais (1994) have already established that CYP2C19*2 and CYP2C19*3 are associated with a poor metabolizing phenotype in humans. According to the available literature, based on studies of nonhuman primates and rodents, environmental contaminants such as tetrachlorodibenzo-*p*-dioxin (TCDD) as well as dioxin-like chemicals may affect the pathophysiology of endometriosis by stimulating chronic inflammation (Rier, 2008). However, there is a paucity of data as to whether human subjects with endometriosis are unable to fully metabolize possible environmental toxicity that may cause susceptibility, when compared with healthy controls.

In connection with endometriosis, although the presence of a polymorphism has been widely examined (Falconer et al., 2007), there has been no study evaluating CYP2C19 gene polymorphisms in women with endometriosis compared with healthy controls. The current study aimed to assess whether the CYP17 T>C polymorphism and polymorphisms CYP2C19*2 and CYP2C19*3 of CYP2C19 are associated with endometriosis when compared with healthy controls, who were confirmed to be without endometriosis by laparoscopy.

Materials and methods

Subjects

Patients undergoing surgery due to a known diagnosis of endometrioma or cases found to have incidental endometriotic lesions during the diagnostic laparoscopy were recruited as the endometriosis group (n = 50). The diagnosis was histologically confirmed by examining the endometrioma cyst walls and patients were classified according to the revised American Fertility Society classification of endometriosis (American Fertility Society, 1985). However, four individuals were excluded from the analysis due to smoking or intake of any medication in the previous 6 months. Of the 46 cases, the numbers of cases with Class I, II, III and IV endometriosis were seven (15.2%), six (13.0%), 26 (56.5%) and seven (15.2%).

Patients free from endometriotic lesions during the surgery were enrolled as controls (n = 43). Of the 43 cases, four cases were excluded from the statistical analysis due to smoking or known drug intake in the previous six months. In the control group (n = 39), diagnostic or surgical laparoscopy was proposed in order to investigate benign ovarian cysts but not endometrioma (n = 3), ectopic pregnancy (n = 8), chronic pelvic pain (n = 7), infertility (n = 5), hydrosalpinx (n = 3), tubal ligation (n = 11) or other reasons (n = 2). In both groups, individuals with leiomyoma, adenomyosis or any history of breast disease and endometrial hyperplasia/carcinoma were excluded. With the exception of six cases among the controls and one in the endometriosis group undergoing laparotomy, all the surgical procedures were performed via laparoscopy.

Genotyping of CYP2C19 and CYP17

A sample of peripheral blood was collected by venipuncture to perform DNA extraction and analysis in each patient. DNA of subjects were isolated from leukocytes by High Pure Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) and stored at −20°C until all subjects of the study were completed. In order to genotype CYP2C19, 5 μl of isolated genomic DNA from human blood were mixed with 10.7 μl H_2O polymerase chain reaction (PCR)-grade, 2 μl Lightcycler CYP2C19 mutation detection mix, 2 μl Lightcycler CYP2C19 reaction mix, and 0.3 μl Lightcycler CYP2C19 enzyme solution. Each capillary was sealed with a stopper and centrifuged at 700*g* for 5 s. The genotypes were detected by LightCycler CYP2C19 mutation detection kit by real-time PCR with a LightCycler instrument (Roche Diagnostics) under the following cycling conditions: pre-incubation at 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 10 s and extension at 65°C for 15 s. In the section of melting-curve analysis, samples were heated from 35°C to 80°C at a rate of 0.2°C/s and the melting peaks of all samples were determined and analysed. The alleles of CYP2C*1 (wild type), CYP2C19*1/CYP2C19*3 (heterozygous) and CYP2C19*3 (mutant) had specific melting peak values at 62°C, 53.5°C/63°C and 53.5°C, respectively. The figures for CYP2C*1 (wild type), CYP2C19*1/CYP2C19*2 (heterozygous) and CYP2C19*2 (mutant) were 60.5°C, 52°C/60.5°C and 52°C.