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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.

To genotype CYP17, the following cycling conditions were used: pre-incubation at 95°C for 10 min, followed by 45 cycles denaturation at 95°C for 10 s, annealing at 55°C for 10 s, and extension at 72°C for 12 s. In the section of melting-curve analysis, samples were heated from 50°C to 85°C at a rate of 0.1°C/s and the melting peaks of all samples were determined and analysed. The alleles of A1A1 (T/T), A1A2 (T/C) and A2A2 (C/C) had specific melting peak values at 63°C, 54°C/63°C and 63°C, respectively.

Statistical analyses

All parameters were given as mean ± SD. Kolmogorov–Smirnov test confirmed the normality of the distribution of the variables. Normally distributed parametric variables were tested by independent Student's *t*-test. Non-normally distributed metric variables were analysed by Mann–Whitney *U*-test, if needed. Chi-squared test and Fisher's exact test were used to analyse nominal variables in the form of frequency tables. Pearson's correlation test was used for correlation analyses. $P < 0.05$ was considered statistically significant. Data analysis was performed using the Statistics Package for Social Sciences version 13.0 (SPSS, Chicago, IL, USA).

Results

The mean ages of the females in the endometriosis and control groups were 34.7 ± 7.1 and 31.7 ± 5.3 years, respectively.

The rate of wild-type CYP17 gene was found to be significantly higher in controls when compared with patients with endometriosis (46.2% and 19.6%, **Table 1**). Although the presence of the heterozygote type CYP17 mutation was similar between groups, the homozygote type mutation in which both of the alleles were affected was observed to be more frequent in patients with endometriosis (**Table 1**). Of note, the presence of any type of CYP17 mutation associated with one or both alleles was also significantly higher ($P = 0.009$).

The frequency and rate of CYP2C*1, CYP2C*2 and CYP2C*3 are shown in **Table 2**. No differences were determined between patients with and without endometriosis with respect to the presence of any mutation related with CYP2C19 (**Table 2**).

The distribution of each mutation of CYP17 and CYP2C19 with respect to the severity of endometriosis in the study group is presented in **Table 3**. All of the cases having Class IV endometriosis ($n = 7$) revealed a polymorphism (any type), with the highest risk of CYP2C*3; however, the difference did not reach statistical significance probably due to the small sample size (**Table 3**). It may be noteworthy that while 41 of the 46 (89.1%) patients in the endometriosis group were found to be associated with any type of mutation of the current genes, only 22 of 39 (56.4%) controls were found to have any type of mutation related with CYP17 and/or CYP2C19 (data not shown, $P < 0.05$). Also of interest is that a correlation was noted between the severity of endometriosis and any mutation of CY17 and/or CYP2C19 ($r = 0.362$, $P < 0.05$).

Discussion

The results of the current study suggest that patients with endometriosis have an increased risk of the A2A2 mutation, which has been shown to be associated with sex steroid metabolism. No statistically significant association was found between severity of disease and any CYPC19 gene polymorphism. However, eight of the nine cases having CYP2C19*3 polymorphism suffered from moderate/severe endometriosis.

There are a number of studies evaluating the impact of various polymorphisms related with the CYP17 gene (Falconer et al., 2007). Single (A1–A2) nucleotide change in the 5′ region of CYP17, which contains a recognition site for the *MspA*I restriction enzyme, configures a mutant allele (A2) gene and increases circulating oestrogen and androgen concentrations (De Carvalho et al., 2007; Feigelson et al., 1997). While the A1 allele of the CYP17 polymorphism is thought to be associated with an increased risk of prostate cancer and benign prostatic hyperplasia (Habuchi et al., 2000) in males, A2 was found to have a close link with oestrogen-susceptible diseases such as breast cancer (Feigelson et al., 2001; Huang et al., 1999). However, the initial trial investigating whether a polymorphism of CYP17 was linked with the pathobiology of endometriosis had failed (Kado et al., 2002). When compared with 177 control women, Kado et al. (2002) did not notice any correlation with A2 polymorphism either in patients with endometriosis or adenomyosis and/or leiomyoma. Similarly, despite the fact that CYP17* T allele was shown to appear more frequently in cases with endometriosis, Hsieh et al. (2004) did not observe any difference regarding the rate of T/T, T/C and C/C genotypes when only patients with severe endometriosis

Table 1 Distribution of CYP17 gene polymorphism in endometriosis and control groups.

Polymorphism	*Endometriosis group* (n = *46*)	*Control group* (n = *39*)
CYP17 A1A1 (T/T)	9 (19.6)[a]	18 (46.2)
CYP17 A1A2 (T/C)	21 (45.7)	18 (46.2)
CYP17 A2A2 (C/C)	16 (34.8)[b]	3 (7.7)
Any mutation	37 (80.4)[a]	21 (53.8)

Values are number (percentage).
[a]Statistically different from control group ($P = 0.009$).
[b]Statistically different from control group ($P = 0.003$).

Table 2 Distribution of CYP2C19 gene polymorphism in endometriosis and control groups.

Polymorphism	*Endometriosis group* (n = *46*)	*Control group* (n = *39*)
CYP2C*1	36 (78.3)	33 (84.6)
CYP2C19*2	1 (2.2)	–
CYP2C19*3	9 (19.6)	6 (15.4)
Any mutation	10 (21.7)	6 (15.4)

Values are number (percentage). None of the comparisons are statistically significant.

Table 3 The distribution of polymorphisms related with the severity of endometriosis.

Severity of endometriosis	*CYP17*		*CYP2C19*		*Any polymorphism*
	A1A2	*A2A2*	*CYP2C19*2*	*CYP2C19*3*	
Class I (*n* = 7)	4 (57.1)	1 (14.3)	–	1 (14.3)	5 (71.4)
Class II (*n* = 6)	2 (33.3)	1 (16.7)	1 (16.7)	–	4 (66.7)
Class III (*n* = 26)	12 (46.2)	10 (38.5)	–	5 (19.2)	25 (96.2)
Class IV (*n* = 7)	3 (42.9)	4 (57.1)	–	3 (42.9)	7 (100)

Values are number (percentage). None of the comparisons are statistically significant.

(*n* = 119) were compared with healthy women (*n* = 128). However, 1 year after the previous report, the same group (Hsieh et al., 2005) reported that the A1 but not the A2 allele was found to be associated with endometriosis in a similar study design. The following trials (De Carvalho et al., 2007; Juo et al., 2006; Vietri et al., 2008) did not reveal any significant association between susceptibility to endometriosis and A1 and/or A2. The varying results in the available studies may be associated with the criteria for defining healthy controls. While some studies (Hsieh et al., 2004, 2005; Vietri et al., 2008) enrolled only cases as controls who were proven to show no signs of endometriosis via laparoscopy, as in this study's design, the remaining (De Carvalho et al., 2007; Juo et al., 2006; Kado et al., 2002) used clinical features to exclude the presence of endometriosis, such as dysmenorrhoea, dyspareunia and/or pelvic pain. The geographical diversity in which the study is undertaken may also contribute to the inconclusive data. In this regard, since the study centre is a tertiary centre for the health service, patients from various parts of the country are admitted to the department. Unfortunately, the only available data related to the origin of the patients is their place of residence. The primary place of birth of the patients or their parents was not noted, since evaluating the possible relationship between the frequency of CYP17 and CY2C19 gene polymorphisms and geographical diversion was not the primary aim of the current study. In the current study an increased risk of the A2A2 allele in patients with endometriosis was observed when compared with healthy individuals, which was theoretically surmised (Juo et al., 2006; Kado et al., 2002) but not reported earlier.

CYP2C19*2 and CYP2C19*3 are established to be responsible for the poor metabolizing phenotype of the CYP2C19 (Yadav et al., 2008). According to animal studies, endometriosis may also be associated with exposure to certain types of environmental chemicals by stimulating chronic inflammation (Rier, 2008). Although there is a paucity of data regarding the effect of those environmental agents on the risk of endometriosis in human subjects, the exposure of the general human population to such agents has been described in the available literature (Rier, 2008). Therefore, one may postulate that a polymorphism of CYP2C19 gene that decreases the ability to detoxify such chemicals may result in a chronic inflammation on peritoneal surfaces that is thought to be linked to endometriosis (Rier, 2008). In addition, the poor metabolizing ability would also fail to remove cytokines released from neutrophils that arise from the related inflammation. Although eight of nine patients having CYP219*3 gene polymorphism were suffering from Class III or IV staged endometriosis, a larger sample size is required to establish a statistically significant association between gene polymorphism and severity of the disease.

The main limitation of the current study is the small sample size, which may explain the failure to observe a significant relationship between CYP2C19*3 and the severity of endometriosis. However, it is noteworthy that the A2 polymorphism of the CYP17 gene was shown to be associated with a susceptibility to endometriosis that was not reported earlier. The results suggest that, in order to clarify the pathobiology of endometriosis, gene polymorphisms related with both sex steroid metabolism and detoxification of the various environmental chemicals and/or cytokines should be widely examined in such cases with a larger number of subjects.

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