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Association of *CYP1A1* (cytochrome P450) *Msp*I polymorphism in women with endometriosis

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ABSTRACT. Endometriosis is a disease that affects 10 to 15% of the women of reproductive age. It is characterized by the presence of endometrial-like tissues outside of the uterus. Some definitions claim that the functional ectopic tissue is sensitive to the action of hormones. Severity of endometriosis is defined according to a system proposed by the American Society for Reproductive Medicine, which is based on laparoscopic findings. A large number of genetic polymorphisms has been reported for *CYP1A1*, the gene that is responsible for enzymes

involved in stage I detoxification of xenobiotics; this gene is located at 15q22-24, and encodes an isoenzyme that catalyzes the oxidation of polycyclic aromatic hydrocarbons present in phenolic compounds and epoxides. The aim of this study was to analyze the frequency of the *Msp*I polymorphism and its relation to endometriosis. We obtained peripheral blood samples from 52 women with endometriosis (confirmed by laparoscopy) as well as 42 women without endometriosis (control group). In the case group, the women were between 25 and 35 years of age; the age range was between 25 and 57 years old in the control group. Molecular analysis was performed by polymerase chain reaction. We found a significant association (P = 0.039) between the polymorphic allele m1 and endometriosis (32.70%). In conclusion, this study showed that the m1 polymorphism is associated with endometriosis, and that W1/m1 and m1/m1 polymorphisms are more frequently observed in patients with infertility and severe endometriosis.

Key words: Endometriosis; *CYP1A* gene; *Msp*I polymorphism; Infertility

INTRODUCTION

Endometriosis is a disease that affects women of reproductive age. This disease is characterized by the presence of endometrial-like tissues (endometrial or stromal glands) outside the uterus, which are also sensitive to the actions of hormones. Endometriosis affects 10 to 15% of women of reproductive age (Guo and Wang, 2006; Abrão et al., 2007; Podgaec et al., 2007).

The most common type of endometriosis occurs in the pelvis, but can also be found in other places such as the central nervous system, the thorax, the urinary tract, the gastrointestinal tract, the extremities and skin tissues, the Pouch of Douglas (behind the uterus), the rectovaginal septum (the tissue between the vagina and the rectum), the Fallopian tubes, the ovaries, the bladder and ligaments, the liver, the spleen, the intestines, and the heart. The major symptoms for this disease are dysmenorrhea, dyspareunia, pelvic pain, and infertility (Abrão, 2000; Podgaec et al., 2007).

The first studies on endometriosis in the last century have been conducted by Sampson in 1927. Based on his clinical experience, Sampson proposed retrograde menstruation as the most probable cause of endometriosis. It was suggested that during menstrual flow, some viable endometrial cells exit the Fallopian tubes and attach to the peritoneal surface, where it can invade the tissue as endometriosis. In addition to this direct transport of cells, this process can also occur via blood, lymph channels, or during surgical wound healing (Kistner, 1983; Bankowski, 2006).

Endometriosis can be diagnosed by histopathology, but laparoscopy is considered the gold standard to confirm the diagnosis (Abrão et al., 2007).

Severity of endometriosis is classified by the American Fertility Society (1985). Disease is considered minimal if only peritoneal spots are present; mild extensive peritoneal spots or adhesions are observed; moderate if the disease is characterized by deep endometriomas; and severe if it shows posterior cul-de-sac obliteration.

The endometriosis classification system is based on the size and depth of ovarian or peritoneal implants, the presence, extent, and type of adhesions, and the degree of cul-de-sac obliterations. For example, in light endometriosis, the ovaries have surface implants without

endometriosis, scar tissues, or pre-ovarian adhesions (Donadio, 2001).

The risk of endometriosis development for women is approximately 0.6-2.0%. This is increased by seven times (4.3-6.9%) if they have first-degree relatives with endometriosis. Genetic predisposition does not seem to play a role in endometriosis; however, some authors have proposed that some specific polymorphisms may be associated with the disease (Kennedy et al., 1995; Arvanitis et al., 2001).

There are many studies indicating that cell detoxification is carried out by enzymes that regulate cell protection. They show that inactivation of such xenobiotics and endogenous toxins enable the preservation of cellular integrity while at the same time inhibit cytotoxic events caused by these substances, which may lead to diseases like endometriosis (Wilkinson and Clapper, 1997).

Detoxification enzymes are classified into two groups based on their functional properties. Phase I enzymes activate the xenobiotics in a more electrophilic state, making them more reactive. Phase II enzymes generally activate following phase I reactions, and inhibit the formation of electrophilic products. In addition, they catalyze the conversion of electrophilic chemicals into inactive conjugates, making them more soluble to facilitate their excretion (Wilkinson and Clapper, 1997).

The best-known examples of phase I/activation enzymes are members of the cytochrome P450 (CYPs) superfamily, which are classified as oxidative enzymes. The most studied phase II enzymes belong to the glutathione-S-transferase superfamily (Cascorbi, 2006).

CYP1A1(OMIM: 108330) is a very important gene for the metabolism of carcinogens. It is located on the long arm of chromosome 15 (15q22-24), and there are several patterns for this gene. The restriction enzyme *Msp*I is produced by a T→C in the non-coding region 3', resulting in a polymorphic allele named m1 (Walker, 1996).

It can be used as a cancer biomarker especially in tumors related to smoking habits, since it is an isoenzyme that catalyzes the oxidation of polycyclic aromatic hydrocarbons into phenolic products and epoxides. This fact suggests that the gene is highly polymorphic due to a thymine/cytosine punctual mutation in the restriction site of *Msp*I. High levels of this enzyme would result in a high capacity to activate polycyclic aromatic hydrocarbons, producing highly reactive electrophilic intermediates that could lead to DNA damage (Cascorbi, 2006).

The aim of this study was to analyze the frequency of the *Msp*I polymorphism and its relation to endometriosis.

MATERIAL AND METHODS

In this study, 94 samples of 10 mL peripheral blood were collected to perform molecular and genetic analyses. The samples were divided into two groups: 52 group I samples were obtained from patients with endometriosis, as confirmed by laparoscopy. This group was further subdivided into 2 subgroups (A and B). Patients in subgroup A (24) complained of infertility and intended to become pregnant; patients in subgroup B (28) did not intend to become pregnant. The inclusion criterion for women in the study was laparoscopic confirmation of endometriosis; those with negative results were excluded. The second group (control) consisted of 42 healthy women without any clinical signs of endometriosis; invasive laparoscopy was not performed in these individuals. The project was approved by the Research Ethics Committee in PUC Goiás (Brazil), and written informed consents were obtained from patients prior to enrolment into the study (0126.0.168.000-08).

Following laparoscopic examination, patients with endometriosis were classified

according to the degree of endometriosis: stage I (minimum), stage II (mild), stage III (moderate), and stage IV (severe).

DNA was extracted from blood using the Wizard genomic DNA purification (Promega®) kit, according to manufacturer instructions. The integrity of the DNA was visualized using a 2% agarose gel.

PCR-RFLP was carried out to detect specific allelic polymorphisms of the *CYP1A1m1* gene. Reactions were carried out in a final volume of 25 μL. The amplified DNA products were subjected to electrophoresis on a 2% agarose gel, and a voltage of 10 V/cm was applied. The DNA bands were stained with ethidium bromide (5 μg/mL), and were analyzed using the VDS® system (Amersham Biosciences, USA). The experiments were performed in duplicate (Carstensen et al., 1993).

PCR-RFLP with *CYP1A1* resulted in a 340-bp amplification product in the wild-type genotype (W1/W1). A single band was produced, because it was not excised by the restriction enzyme *Msp*I. The homozygous mutant genotype (m1/m1) contains a cutting site for the restriction enzyme *Msp*I. As a result, two bands (200 and 140 bp) were produced. The heterozygous genotype (W1/m1) yielded three bands at 340, 200, and 140 bp (Carstensen et al., 1993).

Data were analyzed using statistical calculations of averages and standard deviations, and the Mann-Whitney U-test was used in order to identify significant differences between groups. Frequency analysis was carried out using the chi-square test. The results were analyzed using the statistical program BioEstat® 5.0 (Sociedade Civil Mamirauá/MCT - CNPq). Differences were considered to be significant when $P \leq 0.05$.

RESULTS

The age range of patients (Table 1) with endometriosis was between 25 and 35 years, with an average of 30.23 ± 2.92 years. In the control group, ages ranged between 25 and 57 years, with an average of 37.38 ± 11.36 years. Interestingly, the average age in the control group was almost four times that of the patient group ($P = 0.0004$).

The genotype frequencies of *CYP1A1* in patients with endometriosis (Table 2) for the homozygous wild-type (W1/W1), the heterozygous genotype (W1/m1), and the mutant genotype (m1/m1) were 67.31% (35/52), 28.84% (15/52), and 3.85% (2/52), respectively. The genotype frequencies of *CYP1A1* in the control group for W1/W1, W1/m1, and m1/m1 were 85.71% (36/42), 14.29% (6/42), and 0%, respectively. The results demonstrated that the frequencies of the polymorphic genotypes W1/m1 and m1/m1 were significantly higher in patients with endometriosis as compared with the controls.

Table 1. Average age in patients with endometriosis and controls.

Group	N	Average	SD	P value
Endometriosis patients	52	30.23	2.92	
				0.0004
Controls	42	37.38	11.36	

Analysis performed using the Mann-Whitney U-test.

As shown in Table 3, the frequency of the wild-type allele W1 in *CYP1A1m1* was 67.30% in the endometriosis patients (70/104); the mutant allele (m1) frequency was 32.70% (34/104). In the control subjects, the wild-type (W1) allele frequency was 85.71% (72/84); the

m1 allele (mutant) frequency was 14.29% (12/84). These results suggested that the frequencies of mutant alleles (m1) were higher in women with endometriosis as compared to the control group (P = 0.006)

Table 2. Genotype frequencies of *CYP1A1* in the endometriosis and control groups.

Genotype	Endometriosis [(N) %]	Control [(N) %]	P
W1/W1	(35) 67.31	(36) 85.71	0.087
W1/m1	(15) 28.84	(6) 14.29	
m1/m1	(2) 3.85	(0) 0	
Total	(52) 100	(42) 100	

Analysis performed using the chi-square test.

Table 3. Allele frequencies of *CYP1A1* in the endometriosis and control groups.

Allele	Endometriosis [(N) %]	Control [(N) %]	P value
W1	(70) 67.30	(72) 85.71	0.006
m1	(34) 32.70	(12) 14.29	
Total	(104) 100	(84) 100	

Analysis performed using the chi-square test.

The genotype frequencies of *CYP1A1* (Table 4) in endometriosis type I/II patients were as follows: 68.19% (15/22) of the patients were W1/W1, 31.81% (7/22) were W1/m1, and 0% (0.0/22) was m1/m1. For class III/IV patients, 66.67% (20/30), 26.66% (8/30), and 6.67% (2/30) of the patients corresponded to the W1/W1, W1/m1, and m1/m1 genotypes, respectively. However, the frequencies of m1/m1 and W1/m1 were not significantly different between class III/IV patients and class I/II patients (P = 0.452).

Table 4. Genotype distribution of *CYP1A1* in endometriosis patients (class I/II, class III/IV).

Endometriosis group	Genotype			
	W1/W1 [(N) %]	W1/m1 [(N) %]	m1/m1 [(N)%]	P value
Class I/II (N = 22)	(15) 68.19	(7) 31.81	(0) 0	0.452
Class III/IV (N = 30)	(20) 66.67	(8) 26.66	(2) 6.67	

Analysis performed using the chi-square test.

The genotype frequencies of *CYP1A1m1* (Table 5) in type I/II patients showed that for this class of the disease 68.19% (30/44) of the patients were W1/W1, 31.81% (14/44) were W1/m1, and 0.0% (0/44) was genotype m1/m1. For class III/IV patients with alleles W1/W1 were 66.67% (40/60), 26.66% (16/60) for W1/m1 and 6.67% (04/60) for m1/m1. The frequencies of polymorphic alleles W1/m1 and m1/m1 are more frequent in patients with endometriosis according to the class III or IV compared to patients class I/II, thus the difference is not significant, P = 0.204.

Table 5. Allele frequencies of *CYP1A1* in endometriosis patients (class I/II, class III/IV).

Endometriosis group	Genotype			
	W1/W1 [(N) %]	W1/m1 [(N) %]	m1/m1 [(N) %]	P value
Class I/II (N = 44)	(30) 68.19	(14) 31.81	(0) 0	0.204
Class III/IV (N = 60)	(40) 66.67	(16) 26.66	(4) 6.67	

Analysis performed using the chi-square test.

The genotype frequencies of polymorphism in the *CYP1A1* gene in the fertile patients are shown in Table 6: homozygous wild-type (W1/W1) was 79.16% (19/52), heterozygote (W1/m1) was 16.67% (4/52), and homozygous mutant (m1/m1) was 4.17% (1/52). The genotype frequencies in infertile patients with endometriosis were as follows: homozygous wild-type (W1/W1) was 55.56% (15/27), heterozygous W1/m1 was 40.74% (11/27), homozygous mutant m1/m1 was 3.70% (1/27).

Table 6. Frequencies of *CYP1A1* genotype among cases (endometriosis) (fertile and infertile patients) and controls.

Genotype	Fertile [(N) %]	Infertile [(N) %]	Control [(N) %]	P value
W1/W1	(19) 79.16	(15) 55.56	(36) 85.72	
W1/m1	(4) 16.67	(11) 40.74	(6) 14.28	0.05
m1/m1	(1) 4.17	(1) 3.70	(0) 0	
Total	(24) 100	(27) 100	(42) 100	

Analysis performed using the chi-square test.

In control subjects, the genotype frequency of homozygous wild-type (W1/W1) was 85.72% (36/42), the frequency of W1/m1 was 14.28% (6/42), and the frequency of homozygous mutant (m1/m1) was 0% (0/42). The genotype frequencies of the mutant genotype were found to be significantly higher in the infertile patients than in fertile subjects.

To reassess the results of this study, a sensitivity test was conducted. We found the result to be 70% in patients with endometriosis with a specificity value of 37%. The positive-predictive value was 36%, and the negative-predictive value was 42%. Based on the results, we conclude that the *CYP1A1m1* gene is an important biomarker for endometriosis, and may enhance our understanding of pre-dispositions to endometriosis.

DISCUSSION

This study analyzed endometriosis patients between 25 and 35 years of age. The average age of patients with increased risk of infertility was found to be 30.23 years. These results were in accordance with the study by Moura et al. (1999), who reported that the average age at which a diagnosis of endometriosis is established is around 31 years. In addition, Pritts and Taylor (2003) reported that the average age for the diagnosis of endometriosis is 28 years. The analysis of subjects without endometriosis (control group) revealed an average age of 37.38 years, which was significantly older than that of the patient group. Results from this study revealed an association between endometriosis and the m1 allele; two patients with endometriosis were demonstrated to have the homozygous mutant genotype. Nakata et al. (2004) analyzed 25 patients with endometriosis and 25 without the disease (control group). The allele frequencies obtained from the two groups indicated no association between the polymorphic *CYP1A1m1* allele and endometriosis. We found that the mutant allele frequency was higher in women with endometriosis when compared to the control group. However, this difference was not significant. A study conducted in 310 Indian women with endometriosis and 215 controls found no association between endometriosis and *CYP1A1* gene polymorphisms (Babu et al., 2005).

The study also revealed that the genotypes (m1) were not associated with the severity of the disease. There were no differences in genotype frequencies between class I/II patients and class III/IV patients. However, the m1 genotype frequencies were higher in patients with

severe endometriosis. Similar results were also found in fertile women with endometriosis. This suggested that *CYP1A1* gene polymorphisms may also influence disease progression.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Abrão MS (2000). In: Endometriose, uma visão contemporânea. 1ª ed. Revinter, Rio de Janeiro, 257-235.

Abrão MS, Podgaec S and Dias Jr JÁ (2007). Endometriose, a mulher moderna e o Brasil. *Prat. Hosp.* 50: 73-77.

American Fertility Society (1985). Revised American Fertility Society classification of endometriosis: 1985. *Fertil. Steril.* 43: 351-352. http://dx.doi.org/10.1016/S0015-0282(16)48430-X

Arvanitis DA, Goumenou AG, Matalliotakis IM, Koumantakis EE, et al. (2001). Low-penetrance genes are associated with increased susceptibility to endometriosis. *Fertil. Steril.* 76: 1202-1206. http://dx.doi.org/10.1016/S0015-0282(01)02865-5

Babu KA, Reddy NG, Deendayal M, Kennedy S, et al. (2005). GSTM1, GSTT1 and CYP1A1 detoxification gene polymorphisms and their relationship with advanced stages of endometriosis in South Indian women. *Pharmacogenet. Genomics* 15: 167-172. http://dx.doi.org/10.1097/01213011-200503000-00005

Bankowski BJ (2006). Infertility and Assisted Reproductive Technologies. In: The Johns Hopkins manual of gynecology and obstetrics. 2nd edn (Hearne AE, Lambrou NC, Fox HE and Wallah EE, eds.). Lippincott Williams & Wilkins, Philadelphia, 31: 157-159.

Carstensen U, Alexandrie AK, Högstedt B, Rannug A, et al. (1993). B- and T-lymphocyte micronuclei in chimney sweeps with respect to genetic polymorphism for CYP1A1 and GST1 (class Mu). *Mutat. Res.* 289: 187-195. http://dx.doi.org/10.1016/0027-5107(93)90069-R

Cascorbi I (2006). Genetic basis of toxic reactions to drugs and chemicals. *Toxicol. Lett.* 162: 16-28. http://dx.doi.org/10.1016/j.toxlet.2005.10.015

Donadio N (2001). In: Consenso Brasileiro em Videoendoscopia Ginecológica. Artes Médicas, São Paulo.

Guo SW and Wang Y (2006). Sources of heterogeneities in estimating the prevalence of endometriosis in infertile and previously fertile women. *Fertil. Steril.* 86: 1584-1595. http://dx.doi.org/10.1016/j.fertnstert.2006.04.040

Kennedy S, Mardon H and Barlow D (1995). Familial endometriosis. *J. Assist. Reprod. Genet.* 12: 32-34. http://dx.doi.org/10.1007/BF02214126

Kistner RW (1983). In: Tratado de Ginecologia Segunda Edição. Ediciones Toray, Barcelona, 830.

Moura MD, Negreiros TP, Nogueira AA, Ferriani RA, et al. (1999). Avaliação do Tratamento Clínico da Endometriose. *Rev. Bras. Ginecol. Obstet.* 21: 85-90. http://dx.doi.org/10.1590/S0100-72031999000200005

Nakata LC, Goloni-Bertollo EM, dos Santos I, Oliani AH, et al. (2004). Biomarcadores de Susceptibilidade à Endometriosis. *Rev. Bras. Ginecol. Obstet.* 26: 299-304. http://dx.doi.org/10.1590/S0100-72032004000400006

Podgaec S, Abrão MS, Dias Jr JA, Rizzo LV, et al. (2007). Endometriosis: an inflammatory disease with a Th2 immune response component. *Hum. Reprod.* 22: 1373-1379. http://dx.doi.org/10.1093/humrep/del516

Pritts EA and Taylor RN (2003). An evidence-based evaluation of endometriosis-associated infertility. *Endocrinol. Metab. Clin. North Am.* 32: 653-667. http://dx.doi.org/10.1016/S0889-8529(03)00045-8

Sampson JA (1997). Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. *Am. J. Obstet. Gynecol.* 14: 422-469. http://dx.doi.org/10.1016/S0002-9378(15)30003-X

Walker CL (1996). Cancer susceptibility genes and molecular carcinogenesis. American Association for Cancer Research Special Conference: Cancer Susceptibility Genes and Molecular Carcinogenesis. *Mol. Med. Today* 2: 364-365. http://dx.doi.org/10.1016/S1357-4310(96)30076-2

Wilkinson 4th J and Clapper ML (1997). Detoxication enzymes and chemoprevention. *Proc. Soc. Exp. Biol. Med.* 216: 192-200. http://dx.doi.org/10.3181/00379727-216-44169