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GENETICS

CYP2C19 polymorphism increases the risk of endometriosis

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Abstract
Purpose Estrogen metabolizing gene mutations can be associated with defective hormonal signaling leading to disease processes. Endometriosis is an estrogen dependent that can be influenced by defective signaling in the estrogen pathway.
Objectives To evaluate the association of A/G 85952 *CYP2C19* and A/G 937 *HSD17B1* gene polymorphisms with endometriosis through the investigation of a large Brazilian sample of women with endometriosis and a fertile control group.
Methods Five hundred women with endometriosis and 500 women without endometriosis were tested for *CYP2C19* and *HSD17B1* polymorphisms, by *TaqMan* Real Time PCR. The results were statistically analyzed by chi-square, logistic regression and tested for Hardy-Weinberg equilibrium.
Results The comparison of genotype and allelic frequency of *CYP2C19* polymorphism (rs11592737) in patients with endometriosis and control group showed a statistically significant difference (p=0.0203) and for the *HSD17B1* polymorphism (rs605059) differences were not significant (p=0.0687). Comparing the stages I/II and III/IV endometriosis with the control group for the *CYP2C19* we observed p=0.0133 and p=0.0564, respectively, and for *HSD17B1* the values for p=0.4319 and p=0.0667.
Conclusion We observed that *CYP2C19* polymorphism is associated with endometrisis in Brazilian women and can be considered a potential biomarker of the disease.

Keywords Endometriosis · *CYP2C19* · *HSD17B1* · Infertility · Molecular biomarsker

Capsule In a case-control study comprising 500 women with endometriosis and 500 women without the disease we were able to demonstrate a statistically difference considering genotype and allelic frequency of *CYP2C19* polymorphism (rs11592737). Comparing endometriosis cases classified as stages I/II and III/IV with control group for the *CYP2C19* we observed that the polymorphism is more frequent in the cases with stages I/II. Regarding *HSD17B1* polymorphism no association was also found. We concluded that *CYP2C19* polymorphism is associated to endometriosis in Brazilian women and can be considered a potential biomarker of the disease.

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Introduction

Endometriosis is a steroid-dependent condition recognized as the most common cause of female infertility [1, 2]. The disease is defined by the presence of endometrial glands and/or stroma outside the uterine cavity.

The estrogen-dependent growth of endometrial tissue is mediated by aromatase, that is the key of local estrogenic biosynthesis, promoting the conversion of androstenedione to estrone and from testosterone to estradiol [3].

Genetic polymorphisms in genes associated to the estrogen synthesis pathway, as receptors and metabolizing enzymes of the hormone have been associated to interindividual variation in the levels of circulating estrogen [4].

CYP2C19 is an important gene of cytochrome p450 family and encodes an aromatase associated by the estrogen metabolism, including the conversion of estradiol in estrone and the

2α- e 16α metabolites of the hydroxilate of estradiol and estrone[4]. Besides, the *HSD17B1* gene (17β-hydroxysteroid dehydrogenase typo 1), 17β-HSD reductase dehydrogenase is a gene of the short chain superfamily (SDR) [5], produce the enzyme that catalyzes the final step of the estradiol biosynthesis, in other words, the conversion of the estrone in estradiol, which is also expressed in the endometrium [6, 7].

Some studies point to the association between the development of endometriosis and genetic polymorphisms [8–11]. This study aimed to evaluate the frequency of the polymorphisms 85952 A/G (rs 11592737) and 937 A/G (rs 605059) of the *CYP2C19A1* and *HSD17B1* genes respectively, previously associated with endometriosis in other population, in Brazilian women with endometriosis and in the control group and correlate the clinical and genetics findings with the risk of endometriosis, in a search for molecular biomarkers of this popular disease.

Material and methods

Patients

Five hundred women with endometriosis (mean age: 34.3± 4.0 y) from the Endometriosis Outpatient Clinic of the Human Reproduction Service of Faculdade de Medicina do ABC (FMABC) were studied. Women with endometriosis diagnosed by laparoscopy and with histological confirmation of the disease were selected and classified according to the American Society for Reproductive Medicine (ASRM, 1997) [12]. In the endometriosis group, stage of the disease was found to be minimal/mild (stage I and II) and moderate/severe (stage III and IV).

To compose the control group, five hundred of fertile women (mean age: 32.0±4.0 y) previously submitted to tubal ligation and with confirmed absence of endometriosis were called, from the Outpatient Clinic of familial planning from FMABC.

Clinical data and peripheral blood samples were collected only after explaining the objectives of the study and obtaining signed informed consent, as approved by the Research Ethics Committee of Faculdade de Medicina do ABC.

Molecular analysis

Peripheral blood was collected from each patient and control in an EDTA-containing tube. Genomic DNA was extracted from peripheral blood lymphocytes according to Lahiri and Nurnberger (1991) protocol [13]. DNA samples were quantified and diluted to 50 ng/μL. *CYP2C19* 85952 A/G (rs115927737) and *HSD17B1* 937 A/G (rs605059) polymorphisms were identified by Real time PCR (TaqMan® Assay - C__1329162-10, *CYP2C19* gene and C__2350902_10, *HSD17B1* gene), performed in the thermo cycler Step One Real Time PCR System (Applied Biosystems, Carlsbad, California, USA).

Statistical analyses

Statistical analyses were carried out using SPSS for Windows 11.0 (SPSS, Inc., Chicago, IL). The chi-square was used to compare allele and genotype frequencies between groups and to estimate the Hardy-Weinberg equilibrium. The odds ratio (OR) and range with 95 % confidence interval (CI) were calculated for the presence of the reference genotype using a logistic regression model. All p-values were two-tailed, and 95 % confidence intervals (CIs) were calculated. A p-value < 0.05 was considered statistically significant.

Results

Genotypes and allelic distribution of *CYP2C19* 85952 A/G and *HSD17B1* 937 A/G polymorphisms in women with endometriosis and controls are summarized in Table 1.

The AA, AG and GG genotype frequencies of the *CYP2C19* 85952 polymorphism in the endometriosis group were 328 (65.6 %), 141 (28.2 %) and 31 (6.2 %) respectively. In the controls we observed 345 (69.0 %), 142 (28.4 %) and 13 (2.6 %). When endometriosis group was divided according to endometriosis stage, the genotypes frequencies were 145 (68.7 %), 51 (24.1 %) and 15 (7.1 %) among women with minimal/mild endometriosis and 183 (63.3 %), 90 (31.1 %) and 16 (5.6 %) considering the women with moderate/severe endometriosis (Table 1).

For *HSD17B1* 937 polymorphism the genotype frequencies of AA, AG and GG in the endometriosis group were 134 (26.8 %), 221 (44.2 %) and 145 (29.0 %) respectively. In the controls we observed 121 (24.2 %), 257 (51.4 %) and 122 (24.4 %). When patients were divided according to endometriosis stage, the genotypes frequencies found were 54 (24.8 %), 100 (46.1 %) and 63 (29.0 %) among women with minimal/mild endometriosis and 80 (28.3 %), 121 (42.7 %) and 82 (29.0 %) considering the women with moderate/severe endometriosis (Table 1).

Discussion

Endometriosis is an estrogen-dependent disease. Estradiol, which reaches endometriosis by circulation or is locally produced, acts regulating endometriotic tissue's growth. Circumstantial and laboratory evidence strongly support the idea that estradiol is a key hormone in the growth and persistence of endometriotic tissue as well as inflammation and pain

Table 1 Frequency between the genotypes and alleles for the *CYP2C19* and *HSD17B1* for the endometriosis group in women with endometriosis stage I and II and women with endometriosis stage III and IV, comparing with a control group

Population studied	n	*CYP2C19* genotypes			p^a	Alleles		p^b	OR (95 % CI)	p^c
		AA (%)	AG (%)	GG (%)		A (%)	G (%)			
Infertile women with endometriosis	500	65.6 % (328)	28.2 % (141)	6.2 % (31)	**0.0203**	79.7 % (797)	20.3 % (203)	0.0441	1.26 (1.01–1.58)	**0.016**
Endometriosis I/II	211	68.7 % (145)	24,1 % (51)	7.1 % (15)	**0.0133**	80.8 % (341)	19.2 % (81)	0.2778	1.18 (0.88–1.58)	0.938
Endometriosis III/IV	289	63.3 % (183)	31.1 % (90)	5.6 % (16)	**0.0564**	78.8 % (456)	21.2 % (122)	0.0333	1.32 (1.02–1.72)	**0.005**
Controls	500	69 % (345)	28.4 % (142)	2.6 % (13)		83.2 % (832)	16.8 % (168)			0.543
	n	*HSD17B1* genotypes			p^a	Alleles		p^b	OR (95 % CI)	p^c
		AA (%)	AG (%)	GG (%)		A (%)	G (%)			
Infertile women with endometriosis	500	26.8 % (134)	44.2 % (221)	29 % (145)	0.0687	48.9 % (489)	51.1 % (511)	0.6547	1.04 (0.87–1.24)	0.035
Endometriosis I/II	217	24.8 % (54)	46.1 % (100)	29 % (63)	0.3419	47.9 % (208)	52.1 % (226)	0.4922	1.08 (0.86–1.36)	0.822
Endometriosis III/IV	283	28.3 % (80)	42.7 % (121)	29 % (82)	0.0667	49.6 % (281)	50.4 % (285)	0.9233	1.01 (0.82–1.24)	0.527
Controls	500	24.2 % (121)	51.4 % (257)	24.4 % (122)		49.9 % (499)	50.1 % (501)			0.051

OR-odds ratio CI-confidence interval [a] and [b] versus control group [b] versus idiopathic infertile group; sample power > 90 %

associated to endometriosis [14]. Estrogen levels observed in endometriosis lesions are highly correlated with the levels of steroidogenic enzyme aromatase cytochrome P450 including the abnormal expression of enzymes [15].

Trabert et al. (2011) [16] assessed the metabolic pathway genes of sex hormones and their correlation with endometriosis risk. *CYP19A1* and *HSD17B1*, from the aromatase group, were the ones for what a positive correlation endometriosis risk was found. *HSD17B1* was in the borderline for statistical significance as much as in our present study.

Other study, conducted by Painter et al. (2011) [4], evaluated 3223 women with endometriosis, 1190 women without endometriosis and 7060 people from regular population found similar results; *CYP19A1* showed to be statically associated to the disease, corroborating Trabert's and our results.

CYP19A1 and *HSD17B1* encode important enzymes in the metabolic estradiol pathway. A reasonable hypothesis for the observed association is that estradiol and related hormones' levels are largely dependent of genetic control [17]. Recent conflicting studies in the literature [4, 18–20] relate the presence of polymorphisms in the cytochrome p450 gene family and *HSD17B*1 to infertility. *HSD17B1* has a wider tissue distribution, which enhances estrogen production in target tissues, thus increasing the estradiol/estrone thereof, thereby providing an increased concentration for estrogen receptors (ESR1 and ESR2) [21]. In normal tissues, *HSD17B1* is expressed in placenta, ovaries, follicles, uterus and mammary glands; it is also expressed in breast cancer and leiomyoma and may be involved in disease processes in these tissues, due to a local increase of estradiol [22, 23].

Tsuchyia et al. (2005) [18], in a study conducted in Japan, with 79 cases and 59 controls, found association of *HSD17B1* polymorphism with most severe endometriosis stages compared to the control group ($p<0.01$). In our study, we found not a strong association of *HSD17B1* polymorphism but a tendency of association with the stages III and IV of endometriosis ($p=0.0687$). Despite of not achieving statistically significant results, Hardy-Weinberg equilibrium observation showed that *HSD17B1* mutated allele is more frequent in the case group, especially in stages III and IV.

Considering *CYP2C19*, we found a statistically significant difference between the different genotypes and alleles (normal as being in common) of the 85952 A/G polymorphism, demonstrating its association with endometriosis ($p=0.0203$). Hardy-Weinberg equilibrium evaluation showed that there is a disequilibrium concerning the *CYP2C19* mutated allele in the case population, more frequent in stages I/II, reinforcing the role of the allele in disease pathogenesis. The risk associated to the allele presence is around 0.3.

Corroborating the association of *CYP19* with endometriosis, Bukulmez et al., (2008) [24] demonstrated increased levels *CYP19A1* mRNA in cultured human endometrial explants and stromal cells, and in those specimens the

expression correlated well with the inflammatory stage of endometriosis.

The identification of genes involved in the biological process of endometriosis may have implications in the diagnosis, identifying risk groups and therapeutic targets [25]. Literature already confirmed a genetic background for endometriosis development; however environmental factors and immunological factors play an important role for disease establishment. An aggregation of different pathways is necessary for the appropriate understanding of the disease.

Conclusions

We observed that patients with endometriosis compared to the control group have a difference in the incidence of the genotypes and alleles of *CYP2C19* polymorphism, more evident in the stages I and II of endometriosis. Considering *HSD17B1* gene polymorphisms, no statistical differences were observed. According to the results, we infer that in our sample, the *CYP2C19* gene polymorphism is associated with the presence of endometriosis and can be considered a potential biomarker of the disease.

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