CLINICAL SCIENCE

The progins progesterone receptor gene polymorphism is not related to endometriosis-associated infertility or to idiopathic infertility

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OBJECTIVE: This study aimed to determine the frequency of the PROGINS polymorphism in women with endometriosis-associated infertility, in infertile women without endometriosis and in controls.

INTRODUCTION: The human progesterone receptor gene has two isoforms that modulate the biological action of progesterone: isoform A, which is capable of inhibiting the activation of the estrogen receptors, and isoform B, which has the capacity to activate the estrogen receptors. Several polymorphisms have been described for this gene, among which one stands out: a polymorphism named PROGINS, which has been speculated to be related to the genesis of endometriosis by several studies with conflicting results.

METHODS: This was a prospective study that included 148 patients with endometriosis-associated infertility, 50 idiopathic infertile patients and 179 fertile women as controls. The PROGINS polymorphism was studied by PCR.

RESULTS: Genotypes P1P1, P1P2 and P2P2 (P2 representing the PROGINS polymorphism) of the progesterone receptor gene presented frequencies of 93.9%, 5.4% and 0.7%, respectively, in the women with endometriosis-associated infertility ($p = 0.2101$, OR = 0.51, 95% CI = 0.24-1.09); 94.4%, 4.2% and 1.4%, respectively, in the patients with minimal/mild endometriosis ($p = 0.2725$, OR = 0.53, 95% CI = 0.20-1.43); 93.5%, 6.5% and 0%, respectively, among the patients with moderate/severe endometriosis ($p = 0.3679$, OR = 0.49, 95% CI = 0.18-1.31); 86.0%, 14.0% and 0%, respectively, in idiopathic infertile women ($p = 0.8146$, OR = 1.10, 95% CI = 0.46-2.63); and 88.3%, 10.6% and 1.1%, respectively, in the control group.

CONCLUSION: The data suggest that PROGINS is not related either to endometriosis-associated infertility or to idiopathic infertility in the population studied.

KEYWORDS: endometriosis; polymorphism; PROGINS; progesterone receptor gene; infertility.

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INTRODUCTION

Endometriosis is a common disease defined as the growth of endometrial tissue outside the uterine cavity and results in a vast array of gynecological problems including dyspareunia, dysmenorrhea, pelvic pain and infertility.[1,2] Several studies have revealed a large number of genetic markers related to immune, neuroendocrine and reproductive functions present in high frequency among patients with endometriosis, indicating associations between the development of endometriosis and genetic polymorphisms.[3-6]

Progesterone is a potent antagonist of estrogen-induced proliferation in the endometrium and may play a pivotal role in the pathogenesis of endometriosis. The human progesterone receptor gene is located at chromosome 11q22-23 and has two isoforms that modulate the biological action of progesterone: isoform A, which is capable of inhibiting the activation of the estrogen receptors, and isoform B, which has the capacity to activate the estrogen receptors.[7] Several polymorphisms have been described for this gene, among which one stands out: a polymorphism named PROGINS, which arises due to the insertion of an *Alu* element into intron G between exons 7 and 8 of isoform A of the *PR* gene, resulting in an increase of 306 bp in the gene product.[8]

Wieser et al.[9] studied 95 women with endometriosis and 107 women without endometriosis and concluded that the PROGINS polymorphism is associated with susceptibility to endometriosis. Similarly, Lattuada et al.[10] studied the

PROGINS polymorphism in 131 women with endometriosis and a control group of 127 women and confirmed the relationship between this polymorphism and endometriosis. Similar results were also obtained by Carvalho et al.[11] On the other hand, Govindan et al.[12] tested 445 Indian women: 100 had endometriosis, 80 had uterine fibrosis, 157 had breast cancer, and 108 served as a control group. These authors concluded that the PROGINS polymorphism can be considered a risk marker for breast cancer but not for endometriosis or uterine fibrosis.

Thus, the objective of the present study was to determine the frequency of the progesterone receptor gene polymorphism PROGINS in women with endometriosis-associated infertility or idiopathic infertility and in controls.

MATERIAL AND METHODS

Patients

Among the patients of the Human Reproduction Service of the Faculdade de Medicina do ABC (FMABC), 148 patients with endometriosis-associated infertility (mean age: 34.4 $\pm$ 4.2 years) diagnosed by laparoscopy and classified by histological criteria according to the American Society for Reproductive Medicine[13] and 50 idiopathic infertile women (mean age: 35.5 $\pm$ 3.1 y) were selected. In the endometriosis group, 48.0% (71/148) had minimal/mild endometriosis, and 52.0% (77/148) had moderate/severe endometriosis. For the control group, 179 fertile women (mean age: 39.7 $\pm$ 4.8 years) who had undergone tubal ligation, which allowed confirmation of the absence of endometriosis, were selected from the Family Planning Outpatient Clinic of the FMABC.

The cause of infertility was investigated according to the minimum propaedeutic of the infertile couple: hormonal and biochemistry profiles, serum testing, sexually transmitted disease investigations, imaging examinations, investigations of genetic and/or immunological abnormalities, hysterosalpingography, hysteroscopy, laparoscopy (laparoscopy was performed in all women up to 36 years old and in patients over 36 years old when there were symptoms or imaging examinations abnormalities) and seminal analysis. In the absence of abnormalities in any of these exams, the infertility was considered idiopathic. Patients with endometriosis who did not achieve pregnancy after at least six natural or induced cycles following laparoscopy were considered infertile. All women whose partner had masculine factors involved with the infertility were excluded from the study.

Clinical data and peripheral blood samples were collected only after explaining the objectives of the study and obtaining a signed informed consent form, as approved by the local Research Ethics Committee (CEP FMABC No. 293/2007).

Methods

DNA Extraction

Peripheral blood was collect from each patient and control in an EDTA-containing tube. Genomic DNA was extracted from lymphocytes in the peripheral blood using an illustra blood genomicPrep Mini Spin Kit, according to the manufacturer's instructions (GE Healthcare Life Sciences, USA).

PCR

Molecular analysis of the PROGINS progesterone receptor gene polymorphism was performed according to the protocol of Wieser et al.[9] with modifications. The primers used were 5′-GGC AGA AAG CAA AAT AAA AAG A-3′ (forward) and 5′-AAA GTA TTT TCT TGC TAA ATG TC-3′ (reverse).

The PCR reaction was carried out in a final volume of 25 μl, containing 1X buffer, 2.5 mM $MgCl_2$, 0.1 mM of each dNTP, 50 nM of each primer, 1 U of Taq Polymerase (Invitrogen), and 200 ng of DNA. Amplification was performed with an initial denaturation step at 95 ℃ for 7 min, followed by 35 cycles of denaturation at 95 ℃ for 45 sec, annealing at 50 ℃ for 1 min, and extension at 72 ℃ for 1 min and a final extension step at 72 ℃ for 7 min. The amplification product was visualized in a 1% agarose gel under UV light.

The PCR product presented a single band of 149 bp in the homozygous individuals without the mutation, designated as P1P1. The presence of one 149-bp and one 455-bp band indicated heterozygous individuals, who have one allele without the mutation and one allele with the mutation; these individual were designated as P1P2. The presence of a single 455-bp band indicated individuals with the mutation in both alleles, and these individuals were designated as P2P2.

Statistical analysis

Allele and genotype frequencies were compared between groups using the χ^2-test or the Fisher's exact test. All *p*-values were two-tailed, and 95% confidence intervals (CIs) were calculated. A *p*-value < 0.05 was considered statistically significant.

RESULTS

The genotype and allele frequencies of the PROGINS polymorphism in patients with endometriosis-associated infertility or idiopathic infertile patients and in the control group are summarized in Table 1.

The frequencies of genotypes P1P1, P1P2 and P2P2 of the PROGINS polymorphism in the patients with endometriosis-associated infertility were 93.9% (139/148), 5.4% (8/148) and 0.7% (1/148), respectively ($p = 0.2101$). Among the women with minimal/mild endometriosis, 94.4% (67/71) presented the normal homozygous genotype P1P1, 4.2% (3/71) had the heterozygous genotype P1P2, and 1.4% (1/71) showed the mutated homozygous genotype P2P2 ($p = 0.2725$). In the patients with moderate/severe endometriosis, the frequencies of genotypes P1P1, P1P2 and P2P2 were 93.5% (72/77), 6.5% (5/77), and 0%, respectively ($p = 0.3679$). In idiopathic infertile women, the genotypes P1P1, P1P2 and P2P2 were observed in 86.0% (43/50), 14.0% (7/50) and 0% of women, respectively ($p = 0.8146$). In the control group, 88.3% (158/179) presented the normal homozygous genotype P1P1, 10.6% showed (19/179) the heterozygous genotype P1P2, and 1.1% (2/179) had the homozygous mutated genotype (Table 1).

Considering the alleles, allele P1 was present in 96.6% of patients with endometriosis-associated infertility, in 96.5% of women with minimal/mild endometriosis, in 96.8% patients with moderate/severe endometriosis, in 93.0% of idiopathic infertile women and in 93.6% of the control group. Allele P2, on the other hand, was present in 3.4% of patients with endometriosis-associated infertility ($p = 0.1117$, OR = 0.51, 95% CI = 0.24-1.09), in 3.5% of women with minimal/mild endometriosis ($p = 0.2899$, OR = 0.53,

Table 1 - Frequency of genotypes and alleles of the PROGINS progesterone receptor gene polymorphism in patients with endometriosis-associated infertility, idiopathic infertile patients and in controls.

Population studied	n	PROGINS genotypes			p values	Alleles		p values	OR (95% CI)
		P1P1 (%)	P1P2 (%)	P2P2 (%)		P1 (%)	P2 (%)		
Endometriosis-associated infertility patients	148	139 (93.9)	8 (5.40)	1 (0.7)	0.2101	286 (96.6)	10 (3.4)	0.1117	0.51 (0.24-1.09)
Minimal/mild endometriosis	71	67 (94.4)	3 (4.2)	1 (1.4)	0.2725	137 (96.5)	5 (3.5)	0.2899	0.53 (0.20-1.43)
Moderate/severe endometriosis	77	72 (93.5)	5 (6.5)	0 (0.0)	0.3679	149 (96.8)	5 (3.2)	0.2161	0.49 (0.18-1.31)
Idiopathic infertile patients	50	43 (86.0)	7 (14.0)	0 (0.0)	0.6126	93 (93.0)	7 (7.0)	1.000	1.10 (0.46-2.63)
Controls	**179**	**158 (88.3)**	**19 (10.6)**	**2 (1.1)**		**335 (93.6)**	**23 (6.4)**		

OR = odds ratio CI = confidence interval

95% CI = 0.20-1.43), in 3.2% patients with moderate/severe endometriosis (p = 0.2161, OR = 0.49, 95% CI = 0.18-1.31), in 7.0% of idiopathic infertile women (p = 1.0, OR = 1.10, 95% CI = 0.46-2.63) and in 6.4% of the control group.

The power calculations for the groups of women with endometriosis-associated infertility and idiopathic infertility were 0.80, $\alpha = 0.05$, and <0.50, $\alpha = 0.05$, respectively.

DISCUSSION

The PROGINS polymorphism produces a decrease in the stability of the progesterone receptor gene, causing the receptor to lose its capacity to inhibit the activation of the estrogen receptors. This in turn results in an inadequate control of these receptors, thus rendering the endometrium more vulnerable to the action of estrogen. It is believed that isoform A can lead to increased expression of isoform B (which is responsible for the activation of the estrogen receptors) when it includes the PROGINS polymorphism, thereby contributing to a higher oncogenic action of this polymorphism.[14] Progesterone is involved in the regulation of extracellular matrix metalloproteinases, stimulating the inhibiting factors of these enzymes; it also acts on the expression of angiogenic factors and on cell cycle-regulating factors. Moreover, D'Amora et al.[15] observed that PROGINS variants may influence cell proliferation, viability, and apoptosis in endometrial cell metabolism.

Wieser et al.,[9] Lattuada et al.,[10] and Carvalho et al.[11] demonstrated a significant correlation between the PROGINS polymorphism and endometriosis. On the other hand, Govidan et al.[12] concluded that the PROGINS polymorphism can be considered a risk marker for breast cancer but not for endometriosis or uterine fibrosis. Additionally, van Kaam et al.[16] studied 72 women with endometriosis, 40 women with adenomyosis in the uterine wall, 102 gynecological patients without symptomatic endometriosis and 93 healthy females and concluded that the PROGINS polymorphism does not seem to modify the risk of deep-infiltrating endometriosis. In the present study, the PROGINS polymorphism was present in 6.1% of the patients with endometriosis-associated infertility and in 11.7% of the women in the control group (p = 0.2101). When we studied the patients with stage I/II or stage III/IV endometriosis separately, no statistical difference was found between groups (p = 0.2725 and p = 0.3679, respectively). This finding suggests that the PROGINS polymorphism is not involved in the genesis of the disease in the population studied (Table 2).

Most of the published studies regarding endometriosis have used heterogeneous control groups, such as healthy men and women,[17] newborns,[18] umbilical cord blood[19] and menopausal women.[11] However, the absence of symptomatology in women does not exclude endometriosis, given that 16% of the patients with endometriosis are fertile and asymptomatic;[20] when analyzing newborns and cord blood, it is possible that the subjects will develop the disease in the future; and menopause itself produces regression of the disease in women who may be asymptomatic.

Our control group was carefully selected among fertile and non-menopausal women who had undergone tubal ligation for family planning reasons and who had no sign of endometriosis in their clinical history. Moreover, the surgeon was able to observe while performing the ligation that there were no foci of endometriosis in these women.

Table 2 - PROGINS progesterone receptor gene polymorphisms in different studies.

Study	Population studied	Conclusion of the study
Weiser et al.[8]	95 white women with endometriosis and 107 white women without endometriosis (controls)	PROGINS appears to be associated with endometriosis in white persons
Lattuada et al.[9]	131 Italian women affected by endometriosis and 127 Italian women without laparoscopic evidence of the disease	PROGINS polymorphism of the progesterone receptor may be associated with an increased risk of endometriosis
De Carvalho et al.[10]	121 Women with surgically confirmed endometriosis and 281 controls with normal gynecological exams	PROGINS heterozygosis genotype frequencies were shown to be statistically higher in women with endometriosis than in controls
Govidan et al.[11]	100 Women with endometriosis, 80 women with fibroids and 157 women with breast cancer along with 108 age-matched normal healthy women as controls	PROGINS can be considered to be a predisposing risk marker for breast cancer but not for endometriosis or uterine fibroids
van Kaam *et* al.[15]	72 women with endometriosis, 40 with adenomyosis in the uterine wall, 102 gynecological patients without symptomatic endometriosis and 93 healthy females	The PROGINS polymorphism does not seem to modify the risk of deep-infiltrating endometriosis
Present study	148 women with endometriosis, 50 idiopathic infertile women and 179 fertile women without a history of endometriosis	PROGINS is not related either to endometriosis-associated infertility or to idiopathic infertility in the population studied

This rigorous selection process of the control group may be responsible for the high level of significance of the results obtained.

Clinically, one of the primary concerns regarding endometriosis is its propensity to cause infertility. It is currently estimated that 25% to 50% of women with endometriosis are infertile and that 25% to 30% of all infertile women have endometriotic lesions as the only identifiable cause of their infertility.[1,2] The association between endometriosis and infertility is well established, but the mechanisms responsible for these effects are unknown. Several hypotheses have been proposed to explain how endometriosis causes infertility: 1) pelvic factors, which include adhesions, distort pelvic anatomy,[21] and alter peritoneal function;[22] 2) ovarian factors that stem from endocrine and ovulatory disorders lead to oocyte and embryonic alteration;[23] and 3) uterine factors cause impaired implantation.[24] Pisarska et al.[25] found an increase in the prevalence of PROGINS mutations among 26 women with the diagnosis of unexplained infertility compared to 28 control women (42% vs. 14%); however, in the current study, the PROGINS polymorphism was not associated with infertility (14.0% vs. 11.7%). Our results point to no association between idiopathic infertility and the PROGINS polymorphism; however, the power calculation of the sample was low, suggesting that a larger sample is needed.

It is important to keep in mind that the study was performed in a special group of patients who underwent surgery via video laparoscopy and, after surgery, were exposed for at least twelve months to the possibility of pregnancy, had no male factor involved in the causes of infertility and, nevertheless, did not achieve pregnancy.

CONCLUSION

In conclusion, our data suggest that PROGINS is not related either to endometriosis-associated infertility or to idiopathic infertility in the population studied. However, further studies with much larger samples are needed to confirm the results for the idiopathic infertily group.

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