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Polymorphism of the progesterone receptor gene associated with endometriosis in patients from Goiás, Brazil

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ABSTRACT. We investigated a possible link between endometriosis and polymorphism of the progesterone receptor gene (PROGINS). The endometriosis group consisted of 54 patients with a diagnosis of endometriosis by laparoscopy, and the control group comprised 44 women without endometriosis. Genotypes for PROGINS polymorphisms (A1/A1, A1/A2 and A2/A2) were determined by polymerase chain reaction and analyzed on a 2% agarose gel stained with ethidium bromide. The frequency of polymorphic genotypes (A1/A2 and A2/A2) was significantly higher in patients with endometriosis (33%) than in the control group (16%). We

conclude that there is a significant correlation between PROGINS polymorphism and endometriosis.

Key words: Endometriosis; Progesterone receptor; PROGINS; PCR

INTRODUCTION

Endometriosis is defined as the appearance of foci of endometrial tissue with glandular and/or stromal features identical to the uterine cavity at locations other than the endometrium (Giordano, 1998), showing a strong genetic component (Tempfer et al., 2009). Endometriosis has characteristics of invasion and metastasis, although pathologically, it resembles a benign tumor (Treloar et al., 2005).

It is a painful chronic inflammatory disease, which represents one of the most common benign gynecological disorders. It occurs primarily in women of reproductive age and regresses spontaneously after menopause (Moura et al., 1999; Johnson et al., 2004). Its frequency is 10-15% in women of reproductive age, 3% in postmenopause (Berbel et al., 2008; Hurtado, 2008) and more than 30% in women with fertility problems (Renner et al., 2006).

Clinical suspicions and macroscopic findings in surgery must be confirmed by histological diagnosis. It can affect several organs, so it is actually called multi-systemic, becoming more frequent in the peritoneum and pelvic organs, especially the ovaries, followed by the recto-vaginal septum (Ranney, 1980; Dentillo, 2007). It can manifest itself in many ways, with the most common findings being pelvic pain and infertility (Riachi, 2008).

Macroscopically, the morphology of lesions is quite variable. Multiple shapes and colors characterize the spectrum of endometriosis (Kamergorodsky, 2007).

In 1927, Sampson proposed the theory of retrograde menstruation, describing endometriosis the way it is now known, suggesting that viable endometrial cells shedded after menstruation, reach reflux via the oviducts, the peritoneal cavity with subsequent implantation and local growth (Nakata et al., 2004).

The overexpression of estrogen receptors and progesterone receptor defects are consequences of an abnormal progestational effect on the ectopic and topic endometrium. The exaggerated expression of estrogen receptors and progesterone receptor defects are consequences of the abnormality of the progestational effect on the endometrium and ectopic topic. Changes in its function may facilitate the emergence of the disease because, in the broad sense, they fail to antagonize the proliferative effects of estrogens (Carvalho et al., 2004).

The progesterone receptor gene (PR) in humans is unique and is located on the long arm of chromosome 11, bands 22-23 (11q22-23), where it is responsible for producing two protein isoforms: PR-A and PR-B (Gomes et al., 2006). The antiproliferative effects of progesterone on the endometrium are mediated by the isoform A and activation of isoform B in the absence of type A receptor leads to increased proliferation in the epithelium (Carvalho et al., 2004).

The polymorphism of the progesterone receptor gene (PROGINS) is a complex of three genetic alterations found only in humans. Among them, the PROGINS polymorphism consisting of a 306-bp *Alu* insertion in intron G between exon 7 and 8 of the PR

gene in humans stands out (Donaldson et al., 2002). This insertion would lead to aberrant gene transcription, coding for a variant form of exon 8 and resulting in the inability of the hormone receptor to bind progesterone and to become subsequently activated, with a reduction in final activity mediated by progesterone (Gomes et al., 2006). PROGINS polymorphism is also marked by a missense mutation (substitution of one amino acid for another causing a change in the sequence of the protein) of single nucleotide polymorphism (SNP) in exon 4 and a silent mutation (base substitutions that do not alter the amino acid sequence in the polypeptide chain) SNP in exon 5 (Pearce et al., 2005).

MATERIAL AND METHODS

Ninety-nine peripheral blood samples were divided into two groups, cases and controls, according to the presence or absence of endometriosis. The case group with endometriosis included 54 patients with signs and symptoms suggestive of endometriosis, diagnosed by laparoscopy, and the control group consisted of 45 women without endometriosis. The control group was composed of women who had a mean age over 37 years and were fertile and who did not present clinical evidence of endometriosis by anamnesis or pathology developed mainly during reproductive age (<35 years). They were not subjected to definitive diagnosis, since that involves laparoscopy, a surgical procedure.

Extraction of genomic DNA

DNA was obtained from peripheral blood samples at the Laboratory of the Núcleo de Pesquisas Replicon of the Pontifícia Universidade de Goiás. Genomic DNA was extracted using the Ilustra GFXTM kit (GE Healthcare, USA).

Polymerase chain reaction (PCR)

PCR was performed in a final volume of 25 μL to identify the polymorphism in the PR gene. The conditions for cycling were: initial denaturation at 94°C for 5 min and 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and polymerization at 72°C for 1 min, followed by a final extension at 72°C for 7 min. The following sequences of primers were used to amplify the region containing PROGINS polymorphism in intron G of the PR gene: 5'-GGC AGA AAG CAA AAT AAA AAG A-3' (primer 5') and 5'-AAA GTA TTT TCT TGC TAA ATG TC-3' (primer 3'). The PCR product was subjected to electrophoresis on 2% agarose gels in 1X Tris-borate-EDTA (TBE), stained with ethidium bromide (5 μg/mL), and viewed with the Video Documentation System® VDS (Image Master RV® - Amersham Pharmacia Biotech, USA). The amplification of the region of the PR gene to be studied can generate two separate PCR products: one called A1, of 149 bp, refers to the wild-type allele, i.e., without *Alu* insertion, and another called A2, 455 bp, resulting from the insertion of 306 bp in intron G of the receptor gene. Thus, each patient to be subjected to analysis of two alleles, can be considered homozygous for the wild type (A1/A1) or polymorphic (A2/A2) and heterozygous, containing one of each allele type (A1/A2) (Figure 1).

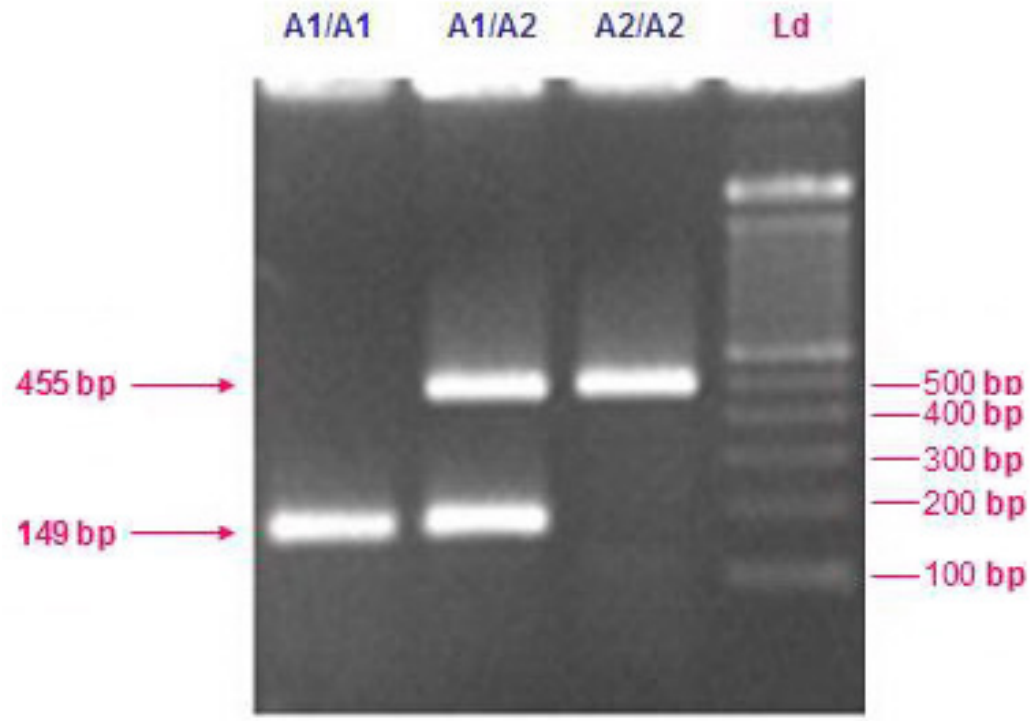

Figure 1. Agarose gel (2%) stained with ethidium bromide showing the possible genotypes for the PROGINS polymorphism: A1/A1 homozygous wild type (149 bp), A1/A2 heterozygote (149 and 455 bp [= 149 bp + *Alu* insertion of 306 bp]) and polymorphic homozygote A2/A2 (455 bp), respectively. *Lane Ld* shows the molecular weight marker 100-bp DNA ladder (Fermentas).

Analysis of results

The genotypes of endometriosis and control groups were compared using the Fisher exact test, with the help of the Bioestat software (version 5.0; biocistron.blogspot.com/).

RESULTS

When analyzing the genotype distribution of the PROGINS polymorphism group with endometriosis (N = 54) we obtained 66.7% for the A1/A1 genotype, 31.5% (17/54) for the A1/A2 genotype and 1.8% (1/54) for the A2/A2 genotype, and 33.3% (18/54) of patients in the endometriosis group had polymorphic genotype for PROGINS (A1/A2+A2/A2).

In the control group (N = 45), 84.5% (38/45) belonged to the A1/A1 genotype, 11.1% (5/45) had the genotype A1/A2 and 4.4% (2/45) had the A2/A2 genotype, and 15.5% (7/45) of these patients had the polymorphic genotype for PROGINS (A1/A2+A2/A2). The frequency of polymorphic genotypes A1/A2+A2/A2 combined was two times higher in patients with endometriosis than in the control group, where this difference was statistically significant (P = 0.0351; see Table 1).

Table 1. Distribution of genotypes A1/A1, A1/A2 and A2/A2 of PROGINS polymorphism in endometriosis and control groups.

	A1/A1		A1/A2		A2/A2		A1/A2+A2/A2		P[1]
	N	%	N	%	N	%	N	%	
Endometriosis (N = 54)	36	66.7	17	31.5	1	1.8	18	33.3	0.0351
Control (N = 45)	38	84.5	5	11.1	2	4.4	7	15.5	

[1]Fisher exact test.

When the endometriosis group was subdivided into fertile and infertile or with regard to the degree of endometriosis (grade I/II and grade III/IV), we did not find statistically significant differences. We also found no significant association between PROGINS polymorphism and personal habits such as physical exercise, smoking, alcohol consumption, and contraceptive use.

DISCUSSION

Due to the increased incidence of endometriosis and the uncertainty regarding their diagnosis and treatment, the search for molecular markers and specific biochemical markers has increased, which would help in the diagnosis and prevention of this disease. Currently, confirmation is made by laparoscopy, an invasive diagnostic procedure that is less aggressive than laparotomy (Abrão et al., 2007).

Our research showed a significant correlation between the insertion of *Alu* (306 bp) in the PR gene and endometriosis, where we observed that 33.3% of patients with endometriosis showed PROGINS polymorphism (A1/A2+A2/A2) and that only 15.5% of patients in the control group showed this polymorphism.

These results run counter to the study conducted in Brazil by Carvalho et al. (2004), which revealed PROGINS polymorphism in 33.0% of endometriosis patients and in 21.0% of the control group. This significance was also observed in an Austrian study conducted by Wieser et al. (2002), in which this change was noted in 31.6% of patients with endometriosis and 15.0% of healthy individuals. Lattuada et al. (2004) conducted a study of Italian women and observed the polymorphism of the progesterone receptor gene in 32.1% of patients with endometriosis and 21.3% of controls, thus in agreement with our findings.

However, studies by Treloar et al. (2005) and van Kaam et al. (2007), conducted in Australia and the Netherlands, respectively, did not find a correlation between PROGINS polymorphism and endometriosis. The results obtained in the Australian and Dutch were different from ours probably due to the different research focus. van Kaam et al. (2007) analyzed the mutation in exon 4 Val660Leu while Treloar et al. (2005) examined eight different types of SNPs in the PR gene and did not use a control group for comparison.

Polygenic inheritance appears to be linked to the development of endometriosis, and the present study focused on the PROGINS polymorphism. This search for specific molecular markers may contribute to the prevention and early diagnosis of this condition. PCR is a method that provides an accuracy of 100%. The analysis for PROGINS in association with endometriosis showed sensitivity of 33% and specificity of 84%, indicating a good test to rule out this pathology. When combined with other methods, its specificity and sensitivity may

increase. Our group is tracking a panel of markers (that are being studied) for molecular diagnosis of endometriosis, preventing normal women from undergoing laparoscopy.

CONCLUSION

Our results indicate a significant correlation between PROGINS polymorphism and endometriosis. The frequency of the polymorphic genotype A1/A2 of the progesterone receptor gene in the group of patients with endometriosis is about three times higher than in the control group. The frequency of genotype A2/A2 of PROGINS polymorphism is about two times higher in the endometriosis group than in the control group. The frequency of polymorphic genotypes A1/A2 and A2/A2 is approximately 2.1 times higher in the endometriosis group than in the control group.

REFERENCES

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

Berbel BT, Podgaec S and Abrão MS (2008). Análise da associação entre o quadro clínico referido pelas pacientes portadoras de endometriose e o local de acometimento da doença. *Rev. Med.* 87: 195-200.

Carvalho CV, D'Amota P, Sato H, Girão MJBC, et al. (2004). Polimorfismo do gene do receptor de progesterona (PROGINS) em mulheres com endometriose pélvica. *RBGO* 26: 613-617.

Dentillo DB (2007). Expressão Gênica Diferencial em Tecido Endometrial Tópico e Lesões Endometrióticas. Doctoral thesis, Faculdade de Medicina de Ribeirão Preto, USP, Ribeirão Preto.

Donaldson CJ, Crapanzano JP, Watson JC, Levine EA, et al. (2002). PROGINS Alu insertion and human genomic diversity. *Mutat. Res.* 501: 137-141.

Giordano MG (1998). Ginecologia Endócrina e da Reprodução. Fundo Editorial BYK, São Paulo, 225.

Gomes MTV, Castro RA, Villanova FE, Silva IDCG, et al. (2006). Relação entre polimorfismo do gene do receptor de progesterona, raça, paridade e ocorrência de leiomioma uterino. *Rev. Bras. Ginecol. Obstet.* 28: 278-284.

Hurtado R (2008). Análise Inter e Intra-Observador no Diagnóstico Anátomo-Patológico de Endometriose. Master's thesis, Faculdade de Medicina, UFMG, Belo Horizente.

Johnson MCP, Pinto CO, Alves ALC, Palomino AA, et al. (2004). P450 arom y microambiente estrogénico en endometrios eutópicos de mujeres con endometriosis. *Rev. Med. Chile* 132: 1475-1482.

Kamergorodsky G (2007). Avaliação da Classificação Histológica da Endometriose Observada em Implantes de Mulheres Portadoras de Endometriose Pélvica Superficial e Profunda. Master's thesis, Faculdade de Ciências Médicas da Santa Casa de São Paulo, São Paulo.

Lattuada D, Viganò P, Somigliana E, Abbiati A, et al. (2004). Analysis of the codon 72 polymorphism of the *TP53* gene in patients with endometriosis. *Mol. Hum. Reprod.* 10: 651-654.

Moura MD, Pereira TN, Nogueira AA, Ferriani RA, et al. (1999). Avaliação do tratamento clínico da endometriose. *Rev. Bras. Ginecol. Obstet.* 21: 85-90.

Nakata LC, Goloni-Bertollo EM, Dos Santos I, Oliani AH, et al. (2004). Biomarcadores de susceptibilidade à endometriosis. *RBGO* 26: 299-304.

Pearce CL, Hirschhorn JN, Wu AH, Burtt NP, et al. (2005). Clarifying the PROGINS allele association in ovarian and breast cancer risk: a haplotype-based analysis. *J. Natl. Cancer Inst.* 97: 51-59.

Ranney B (1980). Endometriosis: Pathogenesis, symptoms, and findings. *Clin. Obstet. Gynecol.* 23: 865-874.

Renner SP, Strick R, Oppelt P, Fasching PA, et al. (2006). Evaluation of clinical parameters and estrogen receptor alpha gene polymorphisms for patients with endometriosis. *Reproduction* 131: 153-161.

Riachi SHMS (2008). Imunoexpressão da Enzima Aromatase p450 em Espécimes Cirúrgicos de Mulheres Portadoras de Endometriose Pélvica Profunda. Master's thesis, Faculdade de Ciências Médicas da Santa Casa de São Paulo, São Paulo.

Sampson JA (1927). Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritonial cavity. *Am. J. Obstet. Gynecol.* 14: 469.

Tempfer CB, Simoni M, Destenaves B and Fauser BC (2009). Functional genetic polymorphisms and female reproductive disorders: part II - endometriosis. *Hum. Reprod. Update* 15: 97-118.

Treloar SA, Zhao ZZ, Armitage T, Duffy DL, et al. (2005). Association between polymorphisms in the progesterone receptor gene and endometriosis. *Mol. Hum. Reprod.* 11: 641-647.

van Kaam KJ, Romano A, Schouten JP, Dunselman GA, et al. (2007). Progesterone receptor polymorphism +331G/A is associated with a decreased risk of deep infiltrating endometriosis. *Hum. Reprod.* 22: 129-135.

Wieser F, Scheneeberger C, Tong D, Tempfer C, et al. (2002). PROGINS receptor gene polymorphism is associated with endometrioses. *Fertil Steril.* 77: 309-312.

