The Progins Progesterone Receptor Gene Polymorphism and Polymorphism of the Estrogen Receptor β Gene in Endometriosis

Amal K. Seleem1, A. A. Abdel Aziz2, E. H. EL.Sayd3, Faeza el Dahtory4, Eatimad A. Basha5*

1(Department of Biochemistry, Faculty of Medicine / Mansoura University, Mansoura, Egypt)
2(Department of Gynecology, Faculty of Medicine/ Mansoura University, Mansoura, Egypt)
3(Department of Chemistry, Faculty of Science/ Port Said University, Port Said, Egypt)
4(Department of Gynocology, Children Hospital, Mansoura university, Mansora, Egypt)
5(Department of Genetic, Children Hospital, Mansoura university, Mansora, Egypt)

Abstract:
Objective: To determine the frequency of the PROGINS polymorphism and the estrogen receptor b gene (ERβ) +1730 G/A polymorphism in infertile women with endometriosis and fertile women.

Subjects and methods: Case-control study included 50 women with endometriosis, and 24 fertile women as control. The PROGINS polymorphism and the ERβ gene +1730 G/A polymorphism were identified by Restriction Fragment Length Polymorphism – Polymerase Chain Reaction (RFLP-PCR).

Results: Genotypes A1A1, A1A2 and A2A2 (A2 representing the PROGINS polymorphism) of the progesterone receptor gene presented frequencies of 88%, 10 %and 2%, respectively, in the women with endometriosis. The allelic frequency of the PROGINS polymorphism was lower in women with endometriosis (P > 0.05) compared with healthy females in the patients with minimal/mild endometriosis (p = 0.978), 84.4 %, 12.5 % and 3.1 %, respectively, among the patients with moderate/severe endometriosis (p = 0.595); 94.4%, 5.6% and 0.0%, respectively, and 83.3%, 12.5 % and 4.2%, respectively, in the control group. Genotypes GG, GA and AA of the ERβ gene presented frequencies of 56%,34% and 10%, respectively, in the women with endometriosis (p < 0.05). In the control group: 91.8 % presented the normal homozygous genotype GG, 8.2% the GA heterozygous genotype and 0.0 % the homozygous mutated genotype AA.

Conclusion: The data suggest that the estrogen receptor gene (ERβ) +1730 G/A polymorphism may be associated with risk of endometriosis. In contrast, the PROGINS polymorphism does not seem to modify the risk of developing endometriosis.

Key words: Endometriosis, polymorphism, PROGINS, estrogen receptor β.

I. Introduction

Endometriosis is a steroid-dependent condition in which a tissue that is histologically similar to the endometrium with glands and stroma grows outside the uterine cavity and becomes implanted in tissues and organs such as the Fallopian tubes, ovaries, peritoneum, colon, the retrovaginal region and the bladder [1], being able to cause pelvic pain, dysmenorrhea and infertility [2]. Endometriosis affects 3%-10% of women in their reproductive years and 20%-50% of women with infertility [3]. Susceptibility to endometriosis depends on a complex interaction of immunologic, genetic and hormonal factors [4]. Many aspects of the female reproductive function are strongly influenced by genetic factors, and numerous studies have attempted to identify susceptibility genes for disorders affecting female fertility such as endometriosis [5]. 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 (PR) 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 [6]. 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 [7]. The estrogen receptor (ER) plays an important role in mediating estrogen action on target tissues. There are two isoforms of estrogen receptors, ERα and ERβ, which are encoded by different genes [8]. ERα has a higher affinity for estrogen and is the predominant form in normal endometrium. Because large amounts of ERβ messenger ribonucleic acid (mRNA) are found in ovaries and granulosa cells, ERβ is likely to play a role in the ovulatory function [9]. Previous studies have demonstrated that both ERα and ERβ are expressed in human endometriotic tissues [10], but the distribution of the isoforms is different between eutopic endometrium and ovarian endometriotic tissues [11]. The +1730 G/A polymorphism in the ERβ gene was associated with an
increased risk of developing endometriosis, regardless of the stage of the disease [12, 13]. Thus, the objective of
the present study was to determine the frequency of the progesterone receptor gene polymorphism PROGINS
and the +1730 G/A polymorphism of the ERβ gene in women with endometriosis and in control group.

II. Material and Methods

II.1 Patients: In the present study, 50 patients with endometriosis-associated infertility were collected from Internal
Medicine University Hospital, Mansoura University, Mansoura, Egypt, (mean age: 30.7±4.3 years) diagnosed
by laparoscopy and classified by histological criteria according to the American Society for Reproductive
Medicine. In the endometriosis group, 36.0% of the patients (32/50) had minimal or mild (stage I/II) and 34.0%
(18/50) had moderate or severe (stage III/IV) endometriosis. For the control group, 24 fertile women (mean age:
31.4±4.1 years) who had undergone tubal ligation, which allowed confirmation of the absence of endometriosis.
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.

II.2 Methods:
DNA Extraction: Genomic DNA was isolated from fresh peripheral blood collected in EDTA tubes using
Thermo Scientific Gene JET Whole Blood Genomic DNA Purification Mini Kit (Fermentas spin columns,
Canada).
P:PCR:
PROGINS detection: Molecular analysis of the PROGINS progesterone receptor gene polymorphism was
performed according to the protocol of Wieser et al.[14] with modifications. The primers used were 5'GGA
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 MgCl2,
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 °C for 7 min, followed by 35 cycles of
denaturation at 95 °C for 45 sec, annealing at 50 °C for 1 min, and extension at 72 °C for 1 min and a final
extension step at 72 °C for 7 min. The amplification product was visualized in a 1.5% agarose gel under UV
light. The PCR product presented a single band of 149 bp in the homozygous individuals without the mutation,
designated as A1A1. 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
A1A2. The presence of a single 455 bp band indicated individuals with the mutation in both alleles, and these
individuals were designated as A2A2(Fig.1).

+1730 G/A OF ERβ detection: A similar approach was used for the detection of the +313G/A polymorphism
and +1730 G/A polymorphism. The primers used were: 5'-TTTTGTCCCCATAGTAACA- 3' (forward) and
5'-AATGAGGGACCAGCA- 3' (reverse). A G/A exchange at nucleotide 1730 in exon 8 introduces a
recognition site for AluI. Digestion by AluI produces one band of 307 bp in the normal ERβ sequence (GG);
The Progins Progesterone Receptor Gene Polymorphism and Polymorphism of the Estrogen ...

three separate bands of 307, 240, and 67 bp, respectively, in the heterozygous polymorphism (GA); and two separate bands of 240 and 67 bp, respectively in the homozygous polymorphism (AA) (Fig.2).

Figure(2): Agarose gel electrophoretic analysis +1730 G/A OF ER-β Polymorphism after AluI digestion analysis: Lane (M) represents the molecular marker (DNA molecular weight marker, purchased from promega Tecnical Service,Catalog#G3161). Lanes (1&4) represents G/G genotype that is presented by one band at 307 bp. Lanes (2&6) represents the G/A genotype showing three bands at 307,240 &67 bp . Lane (3) represents the AA genotype showing two bands 240&67bp. Lane (5): represent G/A genotype but the third band at 67 is not clearly seen.Lane (7) represent the negative control.

II.3 Statistical analysis: Allele and genotype frequencies were compared between groups using the x²-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.

III. Results

PROGINS: Results are summarized in Table 1 and figures 3&4. The allele A1 homozygote (A1/A1) was the dominating genotype in the group of women with infiltrating endometriosis (88%) and 83.3% in the control group. The overall frequency of the PROGINS complex of genomic alterations (A1/A2 heterozygote + A2/A2, homozygote) was 12% of patients with endometriosis and 16.7% in the control group. The A2/A2 homozygous PROGINS genotype was found in 2% of women with endometriosis and 4.2% of women in the control group. None of the observed differences were statistically significant. When the endometriosis group was subdivided with regard to the degree of endometriosis (grade I/II and grade III/IV), we did not find statistically significant differences (Table 2 and Fig. 5&6). We also found no significant association between PROGINS polymorphism and personal habits such as physical exercise, smoking, alcohol consumption, and contraceptive use. Considering the alleles, allele A1 was present in 93% of patients with endometriosis-associated infertility, in 90% of women with minimal/mild endometriosis, in 97.2% patients with moderate/severe endometriosis and in 89.3% of the control group. Allele A2, on the other hand, was present in 7% of patients with endometriosis-associated infertility,(p = 0.526, OR= 0.65, 95% CI = 0.19-2.16), in 9.4% of women with minimal/mild endometriosis (p =0.990, OR= 0.89, 95% CI = 0.25-3.11), in 2.8 % patients with moderate/severe endometriosis (p = 0.231, OR= 0.24, 95% CI = 0.03-2.20), and in 10.4 % of the control group. The control group and infertile patients with endometriosis were in Hardy-Weinberg equilibrium (HWE). The A2/A2 homozygous of the ERβ+1730 G/A polymorphism was found in 10% of women with endometriosis and 0.0 % in control group. Regarding the alleles, allele G was present in 73% of the patients with endometriosis and in 91.8% of the controls, whereas allele A was present in 27% and 8.2% respectively, showing a significant difference in the Allelic distribution related to control group (p = 0.0007, OR= 8.51, 95% CI = 1.93-37.48), in 23.44% of women with minimal/mild endometriosis (p = 0.017, OR= 6.44, 95% CI = 1.39-29.88) and in 36.1% patients with moderate/severe endometriosis (p = 0.0005, OR= 13.00, 95% CI = 2.70-62.53) as shown in table 4 and Fig. 9&10. The control group was in Hardy-Weinberg equilibrium (HWE). The group of infertile patients with endometriosis was not in HWE.

Table 1. Genotype and allele frequencies of the PROINS polymorphism in endometriosis patients and controls.

www.iosrjournals.org 18 | Page
The Progins Progesterone Receptor Gene Polymorphism and Polymorphism of the Estrogen...

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients group</th>
<th>Control group</th>
<th>X²</th>
<th>P</th>
<th>Odd Ratio (OR)(95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1/A1</td>
<td>44(88%)</td>
<td>20 (83.3%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1/A2</td>
<td>5 (10%)</td>
<td>3(12.5%)</td>
<td>0.002</td>
<td>0.660</td>
<td>0.76(0.17-3.48)</td>
</tr>
<tr>
<td>A2/A2</td>
<td>1(2%)</td>
<td>1(4.2%)</td>
<td>0.044</td>
<td>0.538</td>
<td>0.45 (0.03-7.64)</td>
</tr>
<tr>
<td>A1/A2 + A2/A2</td>
<td>6(12%)</td>
<td>4(16.7%)</td>
<td>0.035</td>
<td>0.718</td>
<td>0.66(0.17-2.69)</td>
</tr>
<tr>
<td>Total No.</td>
<td>(n=50)</td>
<td>(n=24)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele</th>
<th>Genotype</th>
<th>Controls without endometriosis</th>
<th>Patients group</th>
<th>Genotyping frequencies</th>
<th>Allelic frequencies</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>A1/A1</td>
<td>20(83.3%)</td>
<td>22 (91.8%)</td>
<td></td>
<td>43 (89.3%)</td>
<td>0.89(0.25-3.11)</td>
</tr>
<tr>
<td></td>
<td>A1/A2</td>
<td>3 (12.5%)</td>
<td>2 (8.3%)</td>
<td></td>
<td>58 (90.6%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A2/A2</td>
<td>1 (4.2%)</td>
<td>0 (0.0%)</td>
<td></td>
<td>6 (9.4%)</td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>A1/A1</td>
<td>20(83.3%)</td>
<td>22 (91.8%)</td>
<td></td>
<td>43 (89.3%)</td>
<td>0.89(0.25-3.11)</td>
</tr>
<tr>
<td></td>
<td>A1/A2</td>
<td>3 (12.5%)</td>
<td>2 (8.3%)</td>
<td></td>
<td>58 (90.6%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A2/A2</td>
<td>1 (4.2%)</td>
<td>0 (0.0%)</td>
<td></td>
<td>6 (9.4%)</td>
<td></td>
</tr>
<tr>
<td>Total No.</td>
<td>(n=50)</td>
<td>(n=24)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele</th>
<th>Genotype</th>
<th>Controls without endometriosis</th>
<th>Patients group</th>
<th>Genotyping frequencies</th>
<th>Allelic frequencies</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>GG</td>
<td>22 (91.7%)</td>
<td>20 (83.3%)</td>
<td></td>
<td>46 (93.7%)</td>
<td>6.68(1.39-32.04)</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>2 (8.3%)</td>
<td>2 (8.3%)</td>
<td></td>
<td>50 (76.6%)</td>
<td>8.64 (1.83-40.78)</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td></td>
<td>14 (21.4%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GA + AA</td>
<td>22 (44%)</td>
<td>2 (8.2%)</td>
<td></td>
<td>7.856</td>
<td>8.1(1.93-37.48)</td>
</tr>
<tr>
<td>Total No.</td>
<td>(n=50)</td>
<td>(n=24)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele</th>
<th>Genotype</th>
<th>Controls without endometriosis</th>
<th>Patients group</th>
<th>Genotyping frequencies</th>
<th>Allelic frequencies</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>GG</td>
<td>22 (91.7%)</td>
<td>20 (83.3%)</td>
<td></td>
<td>46 (93.7%)</td>
<td>6.68(1.39-32.04)</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>2 (8.3%)</td>
<td>2 (8.3%)</td>
<td></td>
<td>50 (76.6%)</td>
<td>8.64 (1.83-40.78)</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td></td>
<td>14 (21.4%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GA + AA</td>
<td>22 (44%)</td>
<td>2 (8.2%)</td>
<td></td>
<td>7.856</td>
<td>8.1(1.93-37.48)</td>
</tr>
<tr>
<td>Total No.</td>
<td>(n=50)</td>
<td>(n=24)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| OR  = Odd's ratio, CI= confidence interval, X²= Qui square test and P= Probability |
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[15]. 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. [16] observed that PROGINS...
variants may influence cell proliferation, viability, and apoptosis in endometrial cell metabolism. Wieser et al., [14] Lattuada et al., [17] and Carvalho et al. [18] demonstrated a significant correlation between the PROGINS polymorphism and endometriosis. On the other hand, Govidan et al. [19] concluded that the PROGINS polymorphism can be considered a risk marker for breast cancer but not for endometriosis or uterine fibrosis. Gimenes et al.[20] suggested that PROGINS is not related either to endometriosis-associated infertility or to idiopathic infertility in the population studied. Additionally, van Kaam et al. [21] 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 12% of the patients with endometriosis-associated infertility and in 16.7% of the women in the control group (p = 0.718). When we studied the patients with stage I/II or stage III/IV endometriosis separately, no statistical difference was found between groups (p = 0.978 and p = 0.595, respectively). This finding suggests that the PROGINS polymorphism is not involved in the genesis of the disease in the population studied (Table 1&2). Our results point to no association between endometriosis and the PROGINS polymorphism; however, the power calculation of the sample was low, suggesting that a larger sample is needed.

The ERβ gene is located on chromosome 14q22-24 [22]. Systemic mutation screening of the coding region and part of the 50 and 30 regions of the ERβ gene revealed two common single nucleotide polymorphisms: G/A exchange at nucleotide 1730 in the 30 untranslated region in exon 8, and a silent 1082 G/A transition in exon 5 [23]. The functional significance of the +1730 G/A polymorphism remains to be clarified. Although +1730 G/A polymorphisms in the ERβ gene do not lead to amino acid changes in the ERβ protein, it is possible that these polymorphisms are in linkage disequilibrium with other regulatory sequence variations that may affect gene expression or function [24]. Furthermore, it has been recently reported that genes containing SNPs can cause different structural folds of mRNA [25]. These mRNA variants may possess different biological functions that interact with other cellular components [13]. The Alu RFLP (1730A/G) in the ESR2 gene was linked to an increased risk of stage IV endometriosis in Japanese women [26], but was not shown to influence disease susceptibility in groups of Italian and Korean women [27]. In the present study, there was a statistically significant difference between the group of infertile women with endometriosis compared to the control group, the +1730 G/A polymorphism was present in 44% of the patients with endometriosis-associated infertility and in 8.2% of the women in the control group (p = 0.003). When we studied the patients with stage I/II or stage III/IV endometriosis separately, statistical difference was found between stage I/II and stage III/IV endometriosis groups and control group (p = 0.040, p = 0.003) respectively. 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. It is important to keep in mind that the study was performed in a special group of patients, who were operated on by videolaparoscopy 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. The deviation of genotype distribution of the patient group from HWE provide additional support for an association of the marker locus with endometriosis.

Polygenic inheritance appears to be linked to the development of endometriosis, and the present study focused on the PROGINS polymorphism and the +1730 G/A 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 and the +1730 G/A polymorphism in association with endometriosis showed sensitivity of 12% and 44% respectively and specificity of 83% and 91.8%, 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.

V. Conclusion

In conclusion, we have shown that the presence of the ERβ gene +1730 G/A polymorphism increase the risk of infiltrating endometriosis compared to the +1730 G/G genotype. In contrast, the PROGINS polymorphism does not seem to modify the risk of developing infiltrating endometriosis; however, the power calculation of the sample was low, suggesting that a larger sample is needed.

Acknowledgement

The authors are indebted to all the women who have contributed to this study and their families by answering the questionnaire and donating biological samples. The authors would like to acknowledge and sincerely thank the following people for their help and support: Dr. Mohammad A. Cord (Director of the Central Lab in Damietta, Egypt) and Ramzy. E. Rashed (chemist in Department of Toxicology, Central Lab, Ministry of Health/Damietta, Egypt).
The Progins Progesterone Receptor Gene Polymorphism and Polymorphism of the Estrogen ...

References