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Estrogen receptor αlpha gene (ESR1) *Pvu*II and *Xba*I polymorphisms are associated to metabolic and proinflammatory factors in polycystic ovary syndrome

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is a complex endocrine disorder that involves multiple factors. Although the etiology of PCOS is unknown, there is an involvement of sex steroid hormones in the pathophysiology of this syndrome. Therefore, polymorphisms in genes involved in the action of estrogen may contribute to a woman's susceptibility to PCOS.
Aim: This study aimed to evaluate the association between the polymorphisms *Pvu*II and *Xba*I in the estrogen receptor alpha (*ESR1*) gene and the occurrence of PCOS. The study also aimed to assess the influence of these polymorphisms on the metabolic and inflammatory profiles of women with PCOS.
Material and methods: This case–control study included 99 women with PCOS, diagnosed according to the Rotterdam criteria, and 104 age-matched healthy women. The polymorphisms were evaluated using polymerase chain reaction–restriction fragment length polymorphism.
Results: No association between the *ESR1* gene polymorphisms and the presence of PCOS was observed. However, we found associations between the *Pvu*II polymorphism and C-reactive protein levels, testosterone levels, family history of diabetes, and waist circumference. The *Xba*I polymorphism was associated with fasting glucose and a family history of hypertension.
Conclusion: These polymorphisms are not associated with PCOS development, but they are involved in the phenotype of complications of the syndrome. Therefore, prior knowledge of these genomic variants might contribute to taking preventive measures that could delay the metabolic and reproductive complications commonly seen in women with PCOS.

1. Introduction

Polycystic ovary syndrome (PCOS) is a complex disorder that involves the interaction of environmental and genetic factors. PCOS is the most common endocrine disorder in women, affecting approximately 5–10% of those of reproductive age, and is characterized by metabolic, reproductive, and psychological alterations (Michelmore et al., 1999; Azziz et al., 2006; Farrell and Antoni, 2010). Furthermore, PCOS patients present increased risk for developing metabolic syndrome, dyslipidemia, glucose intolerance, type 2 diabetes mellitus (T2DM), hypertension, and other cardiovascular diseases (Azziz et al., 2006; Cobin, 2013).

The etiology of PCOS is unknown (Azziz et al., 2006; Dunaif, 1995; Franks, 1995), but men and women who are first-degree relatives of women with this syndrome have an increased risk for developing obesity, insulin resistance, and T2DM. However, the mode of inheritance remains unknown, as does the interaction of genetic background with acquired risk factors, such as diet and lifestyle (Legro et al., 2002; Sam et al., 2008).

Estrogens play an important role in the development and functioning of the male and female reproductive systems. They also act on the skeletal system, lipid metabolism, and the maintenance and protection

Abbreviations: BMI, body mass index; CRP, C-reactive protein; dNTP, deoxynucleotide; ESR, encoding estrogen receptor; FSH, follicle stimulating hormone; HOMA, homeostatic model assessment; IGT, impaired glucose tolerance; IR, insulin resistance; LAP, lipid accumulation product; LH, luteinizing hormone; PCOS, polycystic ovary syndrome; T2DM, type 2 diabetes mellitus; UFMG, Federal University of Minas Gerais; WC, waist circumference.

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of the cardiovascular and nervous systems (Corbo et al., 2011). The gene-encoding estrogen receptor (*ESR*) is located on the long arm of chromosome 6 (6q25.1). It contains 8 exons, and its introns have been shown to be highly conserved (Ponglikitmongkol et al., 1988; Molvarec et al., 2007a). Alternative splicing is observed after gene transcription, resulting in different mRNAs, which differ primarily in the 5′ untranslated region (Flouriot et al., 2000; Kos et al., 2001). Two major isoforms of the *ESR* gene have been described: *ESR1* (ERα) and *ESR2* (ERβ), which have distinct patterns of tissue expression (Moore et al., 1998).

The *ESR1* isoform is expressed in various organs, such as the uterus, liver, kidney, and heart, and particularly in the testis and ovary (Tietz, 1998). This receptor is also coexpressed in tissues, such as the mammary gland, epididymis, thyroid, adrenal, and bone, and in certain regions of the brain (Matthews and Gustafsson, 2003).

ESR1 is a nuclear hormone receptor that functions as an activator of transcription factors and consists of 595 amino acids, with a molecular weight of approximately 66 kDa. The estrogen receptor is activated not only by binding to estrogen, but also by binding to several growth factors (Molvarec et al., 2007b). The absence of *ESR1* can be associated with an increase in circulating luteinizing hormone (LH) levels and a loss in the maturation of follicles, resulting in anovulatory follicles and atresia (Schomberg et al., 1999).

The main polymorphisms are located in intron 1 of this gene and consist of the substitution 397T/C, which creates a restriction site for the *Pvu*II (rs2234693) enzyme, and 351A/G, which creates a site for *Xba*I (rs9340799). These polymorphisms have been associated with male and female infertility, breast cancer, osteoporosis, Alzheimer's disease, cardiovascular diseases, hemostatic abnormalities, preeclampsia, increased abdominal circumference, systemic lupus erythematosus, T2DM, age at menarche, and the onset of menopause (Corbo et al., 2011; Molvarec et al., 2007b; Weel et al., 1999; Schuit et al., 2005; Huang et al., 2006; Stavrou et al., 2006; Lu et al., 2009). A possible functional mechanism attributed to the polymorphisms of the *ESR1* gene includes a change in mRNA processing, producing different variants or isoforms of the protein.

Although the etiology of PCOS is unknown, there is an involvement of sex steroid hormones in the pathophysiology of this syndrome. Therefore, polymorphisms in genes involved in the action of estrogen may contribute to a woman's susceptibility to PCOS. Thus, the aims of this study were to evaluate whether the frequency of *Pvu*II and *Xba*I polymorphisms in the *ESR1* gene is altered in women with PCOS, and to investigate the influence of these polymorphisms in metabolic and inflammatory profiles in this population.

2. Material and methods

2.1. Subjects

This case–control study evaluated 99 Brazilian women with PCOS and 104 healthy women without the syndrome; women in both groups were aged 20 to 50 years. Women with PCOS were recruited at the academic hospital of Federal University of Minas Gerais (UFMG) in Belo Horizonte, Brazil, between 2011 and 2013. The control group was recruited from university employees and students in the same period. The study was approved by the Research Ethics Committee of UFMG (CAAE 0379.0.203. 000-11). All participants signed an informed consent form.

The PCOS diagnosis was performed using the Rotterdam criteria (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004), which cites the presence of at least two of three of the following: oligo/amenorrhea and anovulation, clinical or laboratory hyperandrogenism, and micropolycystic ovaries as seen using ultrasound. The presence of 12 or more follicles in the ovary, each measuring 2 mm to 9 mm in diameter, and/or increased ovarian volume (>10 mL) were considered micropolycystic ovary.

Exclusion criteria for both groups included the presence of diabetes mellitus, autoimmune disease, adrenal disease, kidney and liver disease, thyroid disorders, cancer or tumors, acute inflammatory disease, hyperprolactinemia, hypogonadism, and pregnancy. Subjects being treated with the following medications were also excluded: steroidal and nonsteroidal anti-inflammatory agents, isotretinoin, cyclosporine, antiretroviral, insulin, metformin, and hormonal contraceptives.

2.2. Clinical and laboratorial evaluation

Venous blood samples were obtained after the women fasted for 12 h. In PCOS patients, a second blood sample was collected 2 h after 75 g oral glucose load. Serum glucose, lipid profile, C-reactive protein (CRP), follicle stimulating hormone, (FSH) and total testosterone were measured using Vitros kits (Johnson and Johnson, New York). The insulin level was measured using ARCHITECT immunoassay (Abbott, Illinois).

Dyslipidemic women were defined as those currently using lipid-lowering medication or with altered lipid profile, according to the III Brazilian Guidelines on Dyslipidemia and Atherosclerosis Prevention (total cholesterol > 240 mg/dL, LDL-cholesterol > 160 mg/dL, HDL-cholesterol > 40 mg/dL, and triglycerides > 201 mg/dL) (Santos and Sociedade Brasileira De Cardiologia, 2001). Hypertension was defined as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg at the time of interview, or regular use of antihypertensive medication. Metabolic syndrome was classified according to NCEP/ATP III (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001). Impaired glucose tolerance (IGT) was considered as blood glucose at 120 min > 140 mg/dL after 75 g oral glucose load.

The information about family history of hypertension or diabetes was obtained from medical records. Hirsutism was assessed by using the modified Ferriman–Gallwey method (Goodman et al., 2001) through the visual scoring of hair density on the upper lip, chin, chest, back, lower back, upper abdomen, abdomen, arms, and thighs. A scale of 0 to 4 was used; 0 indicates no hair growth and 4 indicates male pattern growth. A single observer classified all individuals to prevent subjectivity.

The lipid accumulation product (LAP) index was calculated by using the formula: [(waist circumference − 58) × (triglycerides)]. Body mass index (BMI) was also categorized into three groups: normal (<25 kg/m^2), overweight (25–29.9 kg/m^2), and obesity (≥30 kg/m^2) — BMI 1; and two groups: normal/overweight (<30 kg/m^2) and obesity (≥30 kg/m^2) — BMI 2. Values of waist circumference (WC) were further divided into two groups: (1) <88 cm and (2) ≥88 cm (Grundy et al., 2005). Homeostatic model assessment (HOMA) for insulin resistance (IR) and beta cell function (Beta) was calculated using the following formulas: IR, fasting glucose × 0.0555 × insulin / 22.5; Beta, (20 × insulin) / (fasting glucose × 0.0555) − 3.5.

2.3. Genotyping

DNA was extracted from whole blood collected in EDTA (BD), using the Genomic DNA Purification Biopur Biometrix kit. The sequence of primers, as well as the conditions for amplification and genotyping of *Pvu*II and *Xba*I polymorphisms, followed the technique previously described (Molvarec et al., 2007b). Briefly, 50 ng genomic DNA was added to PCR buffer, containing 2 mM $MgCl_2$, 0.2 U Taq DNA polymerase (Thermo), 0.2 mM dNTP (Thermo) mix and 1 μM forward and reverse primers (IDT). PCR reactions were run in a Veriti (Applied Biosystems) thermal cycler and consisted of 35 cycles of 30 s at 95 °C, 40 s at 53 °C, and 1 min at 72 °C. The amplified products were digested with *Pvu*II and *Xba*I, and the fragments were separated by electrophoresis on 6% polyacrylamide gel, yielding specific fragment combinations for each genotype. Positive and negative controls were included in the reaction.