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Killer-cell immunoglobulin-like receptors associated with polycystic ovary syndrome

Laise Nayana Sala Elpidio[a], Josiane Bazzo de Alencar[a], Patrícia Yumeko Tsuneto[a], Hugo Vicentin Alves[a], Mariana Trento Toretta[b], Sérgio Ken It Taura[c,d], Jeane Eliete Laguila Visentainer[a,e], Ana Maria Sell[a,e,*]

[a] Postgraduate Program in Biosciences and Physiopathology at the State University of Maringá, Paraná, Brazil
[b] Biologic Science Department, State University of Maringá, Paraná, Brazil
[c] Health Department of Maringá, Paraná, Brazil
[d] The State University of Maringá, Brazil
[e] Laboratory of Immunogenetics, State University of Maringá, Paraná, Brazil

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ABSTRACT

Polycystic ovary syndrome (PCOS) affects the endocrine system and is associated with low-grade inflammation. Natural killer (NK) cells are involved in the defense of the female reproductive tract, folliculogenesis, ovulation and the menstrual cycle. The killer-cell immunoglobulin-like receptors (KIR) on the surface of NK cells modulate the activation and function of these cells after interacting with human leukocyte antigen (HLA) class I ligands. The objective of this study was to evaluate the possible association of the KIR and their HLA ligands with polycystic ovary syndrome.

Methods: Ninety-three patients with PCOS according to the Rotterdam criteria and 104 healthy controls were included in this study. The HLA class I and KIR genotypes were determined using a PCR-SSO technique, rSSO Luminex®. In order to assess whether the distribution of the *HLA* and *KIR* genotypes was in Hardy-Weinberg equilibrium, Arlequin 3.1 software was used. The frequency distributions in the two study groups were compared using the chi-squared statistic with Yates´s correction using Open Epi software.

Results: The higher frequencies of *KIR3DS1-Bw4* (41% *vs.* 19%, $Pc = 0.002$; OR = 2.90) and homozygotic *KIR2DS4-del* (54% *vs.* 26%, $Pc = 0.0002$; OR = 3.316) in patients compared with controls suggest they confer susceptibility to PCOS. A lower frequency of *KIR2DS4-full* was observed in patients (43% vs. 70%, $Pc = 0.0004$, OR = 0.320).

Conclusion: KIR and its HLA ligands were associated with the development of PCOS in the studied population.

1. Introduction

Polycystic ovary syndrome (PCOS) is a complex endocrine disorder of uncertain etiology, whose global prevalence ranges from 4% to 21% in women of reproductive age, depending on the criteria used (Lizneva et al., 2016). In the long run, patients with PCOS are at high risk of type II diabetes mellitus, reproductive dysfunction, gestational complications, cardiovascular diseases, glucose intolerance and cancer (Legro et al., 2013; Peigné and Dewailly, 2014). The estimated cost of diagnosing and treating the syndrome and its morbidities exceeds US$4 million annually (Azziz et al., 2005).

PCOS is a heterogeneous disease. Diagnostic criteria can be used to define 16 different phenotypes (Bellver et al., 2017). However, in general, two of the following three criteria should be met for the diagnosis of PCOS: (i) clinical or biochemical hyperandrogenism, (ii) ovulatory dysfunction, or (iii) polycystic ovaries. This, though, is after the exclusion of other diseases with similar clinical characteristics. The signs and symptoms of PCOS are diverse and include cutaneous

Abbreviations: PCOS, polycystic ovary syndrome; NK, natural killer; uNK, uterine natural killer; KIRs, killer-cell immunoglobulin-like receptors; iKIR, Kir inhibitors; ITIM, tyrosine-based inhibitory motifs; aKIR, Kir activators; ITAM, tyrosine-based activation motifs; HLA, human leukocyte antigen; bp, base pair; KIR2DS4-del, deleted KIR2DS4; KIR2DS4-ful, without deleted KIR2DS4; LH, luteinizing hormone; FSH, follicle stimulating hormone; WHO, World Health Organization; BMI, body mass index; PCR-SSO, polymerase chain reaction-sequence specific oligonucleotides; SAPE, Conjugated Streptavidin/Phycoerythrin; Bw4-80I, Bw4 containing isoleucine at position 80; Bw4-80T, Bw4 containing threonine at position 80; TNF-α, tumor necrosis factor-alpha

* Corresponding author at: Laboratory of Immunogenetics, State University of Maringá, Paraná, Brazil.
E-mail address: anamsell@gmail.com (A.M. Sell).

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manifestations, menstrual irregularities, hirsutism, acne and alopecia (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004; Legro et al., 2013; Lizneva et al., 2016).

Low-grade inflammation is evident in women with PCOS (Zafari Zangeneh et al., 2017). In the inflammatory process, cells in the immune system such as natural killer (NK) cells and macrophages are recruited to the sites of the inflammation. Most of the uterine NK (uNK) cells have a $CD56^{bright}$ CD16- phenotype, are related to the synthesis of cytokines and have limited cytotoxic activity (Mselle et al., 2007; Matteo et al., 2010); these cells are phenotypically identical to the typical NK cells (Gong et al., 2017). In addition to protecting the uterine mucosa against external agents, these cells modulate homeostasis in the female reproductive system and are involved in folliculogenesis as well as in ovulation and menstruation (King, 2000; Trundley and Moffett, 2004; Yang et al., 2011; Berbic and Fraser, 2013; Berbic et al., 2014). In a normal menstrual cycle, the number of NK cells in the proliferative phase is reduced, but increases after ovulation in the secretory phase; a few days before menstruation these cells undergo apoptosis (King, 2000; Trundley and Moffett, 2004; Yang et al., 2011). Thus, NK cells can help the renewal, differentiation and repair of the endometrium through the release of inflammatory factors (Norman and Brännström, 1996; Trundley and Moffett, 2004; Berbic et al., 2014).

Currently, uterine NK cells are considered to be endometrial markers for PCOS (Piltonen, 2016). Unlike women without the disease, patients with PCOS have reduced numbers of NK cells in the secretory phase of the cell cycle and, because of this, disturbances may occur in the effector functions of NK cells (Matteo et al., 2010), resulting in a homeostatic imbalance of the female reproductive tract, culminating in PCOS manifestations such as menstrual deregulation and infertility (Yang et al., 2011; Berbic et al., 2014).

The activation and function of NK cells comes from the balance of signals transmitted between the activating and inhibitory receptors present on their surface. Among them are the killer-cell immunoglobulin-like receptors (KIR) (Middleton and Gonzelez, 2010). KIR molecules have two or three immunoglobulin domains (2D or 3D), and the inhibitory KIR receptors (iKIR) have a long cytoplasmic tail ("L"). Because of their tyrosine-based inhibitory motifs (ITIM), they send inhibitory signals to the NK cells. In contrast, activating KIR receptors (aKIR) have a short cytoplasmic tail ("S"), have tyrosine-based activation motifs (ITAM) and transduce activating signals to NK cells (Robinson et al., 2010).

KIRs are encoded by polymorphic genes located in 19q13.14 and, to date, the following genes have been described – *KIR2DL1*, *KIR2DL2*, *KIR2DL3*, *KIR2DL4*, *KIR2DL5*, *KIR2DS1*, *KIR2DS2*, *KIR2DS3*, *KIR2DS4*, *KIR2DS5*, *KIR3DL1*, *KIR3DL2*, *KIR3DL3* and *KIR3DS1* – as well as two pseudogenes – *KIR2DP1* and *KIR3DP1* (Robinson et al., 2010). KIRs interact with human leukocyte antigen (HLA) class I ligands present on target cells, encoded by genes located on chromosome 6 (Middleton and Gonzelez, 2010). The *KIR2DS4* gene may have a 22-base-pair deletion (bp) in exon 5 and the transcription process is interrupted early, resulting in a truncated protein unsuitable for anchoring on the surface of the NK cell. Thus, the deletion *KIR2DS4* gene (*KIR2DS4-del*) encodes a soluble molecule and the non-deletion gene (*KIR2DS4-full*) transcribes a protein capable of anchoring to the surface of the NK cell (Middleton et al., 2007; Robinson et al., 2010). *KIR* genes are involved in pathologies of the female reproductive system (Giebel et al., 2014).

Although the contribution of *KIR* genes in PCOS is not clear, studies have shown that genes that regulate the action of gonadotropins and those related to insulin receptors are associated with the disease (Chen et al., 2017). More information about the etiology of PCOS and its heterogeneity will facilitate early intervention, and may reduce the high prevalence and high diagnosis and treatment costs of the condition. This study evaluates a possible association of *KIR* genes and their HLA class I ligands with PCOS.

2. Materials and methods

The research was approved and conducted in accordance with the recommendations of the Permanent Ethics Committee of the State University of Maringá COPEP - no. 354/2011. All the women were aware of the research objective and signed the informed consent form.

The study included 93 patients diagnosed with PCOS and 104 controls without the disease, with the same ethnic and geographical origin, unrelated and matched according to age. All the women were residing in the northwest region of the State of Paraná, Southern Brazil (22° 29′30″-26° 42′59″ S and 48° 02′24″-54° 37′38″ W) and were seen at the Maringá basic health units and at the medical outpatient clinic of the State University of Maringá. Due to the great miscegenation of the Brazilian population, the participants were considered of mixed ethnicity, although mainly individuals of Caucasian origin, in line with the general population of Paraná (Probst et al., 2000).

The PCOS diagnosis was based on the Rotterdam 2004 criteria (Rotterdam, 2004) and the presence of at least two of the following symptoms: oligomenorrhea or amenorrhea, weight gain, hirsutism, acne and alopecia. The biochemical criteria were based on the balance between luteinizing hormone (LH) and follicle stimulating hormone (FSH). The differential diagnosis had to exclude prolactin increase, and an ultrasound examination was performed. Patient details recorded included number of pregnancies, childbirths and abortions, weight, height and body mass index (BMI). The control group consisted of women without PCOS, with regular menstrual cycles, without signs and symptoms of hyperandrogenism, without a personal or family history of infertility, and without other chronic diseases of inflammatory origin.

The clinical and biochemical characteristics of PCOS patients and controls are shown in Table 1.

From each participant, about 4 mL of blood was collected in a tube containing EDTA. The DNA was extracted using the commercial kit Biopur® (Biometrix, Brazil). The DNA concentration and quality were analyzed by optical density measurement in a NanoDrop2000® spectrophotometer (Wilmington, USA).

2.1. HLA and KIR typing

Typing for the *KIR* genes and *HLA-A*, *-B* and *-C* alleles and allelic groups were undertaken using the PCR-SSO (Polymerase Chain Reaction-sequence Specific Oligonucleotides), medium-resolution and high-definition technique, using rSSO Luminex® genotyping kits (One Lambda Inc., Canoga Park, CA, USA). The principle of the technique is the amplification of the target DNA by PCR-SSO using specific primer sets. Each PCR product undergoes a denaturation reaction, followed by neutralization. The DNA is biotinylated through hybridization with microsphere-conjugated fluorescent probes specific to the alleles, allowing their detection with the conjugate phycoerythrin-streptavidin

Table 1
Clinical and biochemical characteristics of patients with polycystic ovary syndrome (PCOS) and controls.

Characteristics	PCOS	Controls	*P* value
Age	29.04 (± 8.98)	31.39 (± 7.89)	0.052
Weight	60.26 (± 21.03)	60.57 (± 21.15)	0.918
BMI[1] ≥ 25	29.79 (± 4.09)	29.52 (± 4.24)	0.783
Pregnancies: number	1.06 (± 1.07)	–	–
Abortions :number	0.17 (± 0.43)	–	–
Deliveries: number	0.90 (± 0.95)	–	–
LH[2] (mUI/mL)	12.77 (± 7.48)	–	–
FSH[3] (ng/mL)	5.62 (± 1.89)	–	–
Prolactin (ng/mL)	12.02 (± 4.58)	–	–

Note: Values shown as mean ± standard deviation.
[1] BMI: Body mass index.
[2] LH: Luteinizing hormone.
[3] SH: Follicle stimulant hormone.