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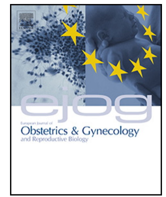

Association of adiponectin and resistin gene polymorphisms in South Indian women with polycystic ovary syndrome

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ABSTRACT

Objectives: To investigate whether genetic polymorphisms in the resistin and adiponectin genes cause a predisposition towards polycystic ovary syndrome (PCOS) in a South Indian women population.
Study design: This case controlled study included samples from 484 study subjects (282 diagnosed with PCOS and 200 normal controls). The clinical and biochemical parameters of the samples assayed included BMI, LH, FSH, testosterone, fasting glucose, adiponectin and resistin levels. Three single nucleotide polymorphisms of the resistin (RETN) gene 420(C→G) (rs1862513), 299(G→A) (rs3745367), and 62(G→A) (rs3745368), and two single nucleotide polymorphisms of the adiponectin (ADPIOQ) gene 45(T→G) (rs2241766), and 276(G→T) (rs1501299), were analyzed using a PCR-RFLP method. Statistical analysis was carried out to determine the association of the genotypic and allelic variations with the syndrome and also analyze the influence of genotypic variations on adipokine levels.
Results: Serum levels of testosterone, LH, fasting glucose and resistin were found to be significantly increased in the PCOS patients when compared to controls, while adiponectin was found to be significantly lower ($P < 0.05$). BMI was found to positively correlate with resistin levels and negatively correlate with adiponectin levels. A positive association was found between the RETN promoter 420 (C→G) SNP and the intron 2 299 (G→A) variant of the resistin gene, while no association was found between the ADPIOQ gene polymorphisms and PCOS. The 'GG' variant of the adiponectin 45 (T→G) variant showed a near-significant tendency towards a decreased concentration of adiponectin in PCOS patients.
Conclusions: Polymorphisms of the resistin gene could be assigned to play a role in increasing the risk of PCOS. However, the adiponectin gene does not seem to play a major role in PCOS susceptibility in a South Indian population. Serum adiponectin and resistin levels were more dependent on BMI rather than the presentation of PCOS. Obesity plays a major role in aggravating the hormonal disturbances found associated with PCOS.

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Introduction

Polycystic ovary syndrome (PCOS) is a 'metabolic malady' commonly observed in adolescent girls with an estimated prevalence of 6% to as high as 26% [1–3]. Several decades after its first description by Stein and Leventhal in 1935, the aetiology of this heterogeneous disorder still remains unclear. PCOS is associated with various metabolic risk factors, of which insulin resistance (IR) and obesity play a central role. The phenotypic expression of women affected with this syndrome are varied with some women being obese, some being lean, some women showing insulin resistance and increased production of adipokines irrespective of their body mass index (BMI) [4].

Adipokines are proteins that are secreted by the adipocytes in the adipose tissue, which have a hormone like function [5]. The adipose tissue plays an important role in the regulation of physiological processes such as reproduction, immune responses, and glucose and lipid metabolisms, all of which are mediated through the production of these adipokines [6]. A few of the adipokines, which are secreted by the adipocytes, include leptin, adiponectin, resistin, visfatin and retinol binding protein-4.

Resistin, a protein identified by Steppan et al. [7] that is secreted by adipocytes, was found to be a strong candidate linking excess

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adiposity to IR. A number of studies have been conducted worldwide to determine the possible association of resistin with PCOS, but no decisive results have been obtained as yet. Seow et al. [8] reported that over-expression of resistin could be a contributing factor to the pathogenesis of PCOS. Genetic association studies on the resistin gene (*RETN*) with PCOS have also given varied results [9–11]. However, its role cannot be eliminated as it is an important adipokine involved in IR, obesity and PCOS [6].

Adiponectin (*ADIPOQ*) was first identified as a 30 kDA protein that was secreted in adipocytes during adipocyte differentiation [12] and is the most abundant adipokine [6]. Unlike resistin, which is over expressed in obesity, the expression of adiponectin mRNA was found to be significantly reduced in obesity [13]. Hypoadiponectinaemia has also been observed in type 2 diabetes mellitus (T2DM) [14]. Adiponectin has been implicated as having a possible role in the regulation of steroidogenesis in PCOS [15,16]. Genetic association studies have mainly focused on two common polymorphisms, one in exon 2 (45 T→G) and one in intron 2 (276 G→T) of the *ADIPOQ* gene and they have been associated with insulin resistance, obesity and risk for T2DM [17–19]. Studies carried out on the association of genetic polymorphisms of the *ADIPOQ* gene with PCOS have yielded diverse results [20,21].

Because adipokines act as a regulatory factor for many critical metabolic functions, any genetic variations in the adipokine genes may alter normal metabolic functions and lead to various metabolic diseases or associated syndromes [22]. Hence, studying the impact of such genetic variations in adipokine genes can determine their association with disease susceptibility in a particular population.

Obesity and T2DM are two of the most common metabolic defects found to be associated with PCOS. Resistin and adiponectin are two candidate genes that have been implicated in obesity related T2DM [7,23]. Studies performed in diverse populations to determine the association of PCOS with resistin and adiponectin have provided varied results based on the ethnicity of the population studied. Hence, we have undertaken a pioneer study to investigate whether the genetic polymorphisms in the *RETN* and *ADIPOQ* genes could cause a predisposition towards PCOS in a South Indian women. In the present study, we analyzed three gene polymorphisms of the *RETN* gene and two gene polymorphisms of the *ADIPOQ* gene (Table 1) and determined whether they were associated with PCOS in South Indian women.

Materials and methods

Study population and patient selection

The study population consisted of 482 South Indian women (282 diagnosed as PCOS and 200 normal female controls). Patients were recruited from a leading women's health centre and private fertility clinic in Tamil Nadu and Kerala between December 2010 and October 2013. PCOS was diagnosed as the presentation of any two of the following three criteria: (i) presence of clinical and/or biochemical signs of hyperandrogenism, (ii) presence of chronic anovulation (less than six cycles in 12 months), and (iii) polycystic ovaries, as recommended by the Rotterdam criteria, 2003 (The Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group, 2004) [24]. Exclusion criteria conditions included Cushing's syndrome, androgen-secreting tumours, hyperprolactinaemia, thyroid dysfunction and enzyme 21-hydroxylase deficiency. Patients who had received prior hormonal therapy were also excluded. A pre-structured questionnaire was provided to the participants and details of their habits and lifestyles were noted. Normal samples were collected randomly from healthy women who had no history of menstrual irregularities, reproductive concerns or hormonal abnormalities. All of the study participants were informed in detail about the study, and written informed consent was obtained. 5 ml of intravenous blood was collected from all of the patients and normal female controls after an overnight fast, at some point within 2–6 days of the menstrual cycle and was stored appropriately. The serum and plasma were isolated and stored at $-20\,^{\circ}$C. Anthropometric measurements of the study population including weight and height were obtained along with their age to determine their BMI. The BMI of each subject was calculated as weight (kg)/height (m^2). The age of the PCOS group was younger than the control group. However, BMI matched groups were generated to limit the possible confounding effects of obesity and the study subjects were further divided into groups based on their BMI: lean ($BMI < 25\ kg/m^2$) and obese ($BMI > 25\ kg/m^2$) for both PCOS and controls. All of the women chosen for the study were genetically unrelated.

Biochemical analysis

Serum reproductive hormones including follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (T) levels of all the study subjects were measured using an enzyme-linked fluorescent immunoassay (ELFA) method using a mini-Vidas device (BioMerieux SA, France) according to the manufacturer's instructions. The fasting glucose levels were analyzed by colorimetric assays using a fully automated biochemistry analyser (Robonik, India). Serum adiponectin and resistin were measured using ELISA kits (RayBiotech, Inc.). The minimum detectable level of adiponectin was 0.025 ng/ml and the intra- and inter-assay coefficients of variation were 10% and 12%, respectively. The minimum detectable level of resistin was 0.002 ng/ml and the intra- and inter-assay coefficients of variation were 10% and 12%, respectively.

Genotype analysis

Genomic DNA was extracted from the patients and controls using a rapid DNA isolation method as described previously by Suganthi et al. [25]. A PCR-RFLP method was used to genotype the *RETN* and *ADIPOQ* genes. PCR products were run on a 2% agarose gel, while the digested products were run on 2% or 3% gels. The *RETN* 62 (G→A) digested products were run on 10% acrylamide gels. The gels were then stained with ethidium bromide and visualized under UV light. The details of the analyzed SNP's and the PCR-RFLP's conditions are presented in Tables 1 and 2.

Statistical analysis

Statistical analysis was performed with the Statistical Package for Social Science for Windows, version 16.0 (IBM SPSS, Chicago, USA). Continuous data were compared between the cases and controls and the analysis was carried out using an unpaired *t*-test, whereas the categorical data, including the genotype, allele frequencies and the deviations from Hardy–Weinberg equilibrium (HWE), were assessed with Pearson's χ^2 test. The odds ratios (ORs)

Table 1
Details of the analyzed SNPs.

Gene studied	SNP ID	Chromosome location	Location of SNP investigated
RETN	rs1862513	19 p13.2	Promoter 420 (C→G) variant
RETN	rs3745368	19 p13.2	3′ UTR (62 G→A) variant
RETN	rs3745367	19p13.2	Intron 2 (299 G→A) variant
ADIPOQ	rs1501299	3q27	Intron 2 (276 G→T) variant
ADIPOQ	rs2241766	3q27	Exon 2 (45 T→G) variant

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