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Short communication

Polymorphisms in the IRS-1 and PPAR-γ genes and their association with polycystic ovary syndrome among South Indian women

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ABSTRACT

Polycystic ovary syndrome is known to be characterized by metabolic abnormalities such as hyperinsulinemia, adiposity and dyslipidemia. Both insulin receptor substrate-1 and peroxisome proliferator-activated receptor-γ have emerged as significant candidate genes in the pathogenesis of PCOS. In this study, we report for the first time, the association pattern of these genes with PCOS among South Indian women. Two hundred fifty PCOS cases and 299 controls were sequenced for IRS-1 exon1 and PPAR-γ exon 2 and exon 6 to study the already reported SNPs in other ethnic groups and to identify any novel SNP in these exonic regions specific to the Indian population. We did not find any novel SNP in our population except for those already reported-two IRS-1 polymorphisms (Gly972Arg and G2323A) and two PPAR-γ polymorphisms (Pro12Ala and His447His). While the IRS-1 polymorphic alleles had a similar distribution between cases and controls, the PPAR-γ exon 2 Ala allele and exon 6 His447His T allele were significantly more in the controls than in the cases ($p \leq 0.05$). Haplotype association analysis also suggests that both IRS-1 and PPAR-γ haplotypes with mutations depicted reduced frequency of hyperandrogenic and metabolic traits in PCOS compared to the haplotype with only wild type alleles. Our study on Indian women suggests that while IRS-1, contrary to the earlier findings in other ethnic groups, seems to have a probable protective role against development of specific PCOS sub-phenotypes, the evidence for a probable protective role of PPAR-γ is reaffirmed in our study.

1. Introduction

Polycystic ovary syndrome (PCOS) is the leading cause of anovulatory infertility with an underlying complex genetic etiology. In addition to the reproductive defects, polycystic ovary syndrome is characterized by significant metabolic abnormalities that include fasting and glucose-stimulated hyperinsulinemia, peripheral insulin resistance (affecting predominantly muscle and adipose tissue), abnormalities of energy expenditure (leading to obesity) and dyslipidemia (Franks et al., 1997). Women with PCOS are, therefore, at a substantially increased risk of developing impaired glucose tolerance and type 2 diabetes mellitus (T2DM) at a younger age. It is established that women with PCOS have profound insulin resistance which is due to a post binding defect of insulin signaling in the classic insulin target tissues, adipocytes and skeletal muscle (Venkatesan et al., 2001). The available evidence suggests that single nucleotide polymorphisms within genes encoding insulin signaling molecules affect insulin resistance and PCOS. Earlier studies have shown that insulin receptor substrate (IRS)-1 Gly972Arg polymorphism affects the tertiary structure of IRS-1 protein, resulting in impaired glucose metabolism and insulin resistance (Almind et al., 1996). Apart from insulin resistance, obesity is also a prominent feature of PCOS and it has been further suggested that obesity contributes to the development of PCOS in some women by exacerbating a preexisting insulin resistance (Nestler, 2000). Since both PCOS and T2DM share certain phenotypic features such as obesity and insulin resistance and the fact that a substantial proportion of women with PCOS are overweight,

Abbreviations: PCOS, polycystic ovary syndrome; IRS-1, insulin receptor substrate-1; PPAR-γ, peroxisome proliferator-activated receptor-γ; T2DM, type-2 diabetes mellitus; TZD, thiazolidinediones; INSR, insulin receptor; ESHRE, European Society of Human Reproduction and Embryology; ASRM, American Society for Reproductive Medicine; DNA, deoxyribose nucleic acid; PCR, polymerase chain reaction; LD, linkage disequilibrium; SNP, single nucleotide polymorphism; HWP, Hardy–Weinberg proportion; BMI, body mass index; WHR, waist hip ratio; SE, standard error; FSH, follicle stimulating hormone; LH, luteinizing hormone; RBS, random blood sugar; HDL, high density lipoprotein; LDL, low density lipoprotein; OR, odds ratio; CI, confidence interval.

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many are obese and some are extremely obese, the peroxisome proliferator-activated receptor-γ (PPAR-γ) gene has been investigated as a candidate in the pathogenesis of PCOS. PPAR-γ is a transcription factor involved in adipogenesis, energy metabolism and a functional receptor for thiazolidinediones (TZDs) introduced as insulin-sensitizing agents. Additionally, PPAR-γ is a candidate gene for the regulation of adipose tissue metabolism in humans and also a susceptibility gene for the development of both obesity and diabetes (Unluturk et al., 2007). Polymorphisms in both these genes – IRS1 and PPAR-γ – have been studied extensively among PCOS cases, though yielding contradictory results. While in IRS-1 gene, the Gly972Arg (G/A) polymorphism is widely studied (Baba et al., 2007; Dilek et al., 2005; Dravecká et al., 2010; Ehrmann et al., 2002; El Mkadem et al., 2001; Ertunc et al., 2005; Lin et al., 2006; Sir-Petermann et al., 2001; Villuendas et al., 2005), there are two PPAR-γ gene polymorphisms, Pro12Ala (C/G) polymorphism in exon 2 and silent His447His (C/T) polymorphism in exon 6, that have been thoroughly investigated in various populations (Antoine et al., 2007; Gu and Baek, 2009; Hara et al., 2002; Koika et al., 2009; Korhonen et al., 2003; Orio et al., 2003, 2004; San-Millán and Escobar-Morreale, 2010; Tok et al., 2005; Wang et al., 2006; Xita et al., 2009).

It is observed that South Asians are more insulin resistant than Caucasians (Wijeyaratne et al., 2002). Moreover, there is growing evidence in support of the hypothesis that Asian Indians, particularly South Indians, as an ethnic group, seem to be particularly predisposed to develop T2DM (Abate and Chandalia, 2007; Mohan et al., 2007). Although, there are recent studies that have investigated the role of different candidate genes in the T2DM pathophysiology (Radha and Mohan, 2007), a complete paucity of such studies exists in India with reference to PCOS, despite sharing pathogenetic factors. In the Indian context, most of the PCOS studies have been confined to the clinical dimensions (Dasgupta and Reddy, 2008). A couple of recent studies, however, deal with an association of single SNP each of CYP11A1, leptin and insulin receptor (INSR) genes with polycystic ovary syndrome (Maitra et al., 2004; Mukherjee et al., 2009). Given the literature implicating the role of IRS-1 and PPAR-γ in the development of insulin resistance and obesity, we investigated these genes for the possible association with PCOS among the South Indian women. In this context, it may be noteworthy that the Indian populations present a totally different pattern of genetic susceptibility to various complex genetic disorders as compared to the populations of other ethnic backgrounds (Aruna et al., 2010; Dhandapany et al., 2009). Therefore, it becomes imperative to study the Indian populations for complex genetic disorders in general and particularly PCOS, to have a more comprehensive understanding on the nature of genetic predisposition in this Asian region.

2. Materials and methods

2.1. Study design

A total of 549 women consisting of 250 PCOS cases (aged 14–40 years) and 299 controls (aged 14–47 years) were enrolled for the present study. Patients were recruited from the Gynecology clinic of the Osmania General Hospital, Hyderabad as well as from an infertility clinic (Anu's Test Tube Baby Centre, Hyderabad) as per the Rotterdam criteria, 2003 (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group) according to which any two of the following three conditions need to be fulfilled for the inclusion: (i) presence of clinical and/or biochemical signs of hyperandrogenism, (ii) infrequent periods with intermenstrual interval of more than 35 days, and (iii) polycystic ovaries; an ovary with the ultrasound appearance of more than 10 subcapsular follicles (<10 mm in diameter) in the presence of prominent ovarian stroma was considered polycystic. Patients with hyperprolactinemia, thyroid and adrenal diseases, 21-hydroxylase deficiency, and androgen-secreting tumors were excluded. The weight and height of the subjects were recorded. Hirsutism was defined as a Ferriman–Gallwey score of more than 5 (Mifsud et al., 2005). Normal controls with no history of treatment for fertility, no evidence of clinical hyperandrogenism (hirsutism/acne/alopecia) and with normal menstrual cycles every 25–32 days were recruited from the family planning center of the Osmania hospital and from the general population.

2.2. Ethics statement

Intravenous blood samples (~5 ml) were collected from both the patients and controls after obtaining their informed written consent. The study protocol was approved by the Indian Statistical Institute Review Committee for Protection of Research Risks to Humans.

2.3. DNA extraction, amplification and sequencing

DNA was extracted from the peripheral blood samples of the patients and control using the phenol-chloroform method (Sambrook et al., 1989). We carried out PCR amplification and direct sequencing to screen the exons of IRS-1 gene (exon 1) and PPAR gamma (exons 2 and 6) in order to validate the already known SNPs in those regions as well as to identify any novel variant that may be specific to Indian population. The IRS-1 exon was amplified and screened using seven overlapping primers. The primer sequences along with the amplification conditions are enlisted in Supplementary Table 1. Each PCR was optimized with respect to the concentration of Mg^{2+} ions. The PCR-mix consisted of 10× PCR Buffer, 10 μM dNTP-mix, 1 μM of each primer, 1 U Taq-polymerase and 40 ng template DNA in a reaction volume of 10 μl. Reactions were carried out in an ABI GeneAmp9700 thermal cycler (Applied Biosystems, Foster City, CA). Forward and reverse primers and annealing temperature are given in Supplementary Table 1.

Cycle sequencing of PCR products were carried out with either the forward or the reverse primers using the Big-Dye Terminator ready reaction kit (Applied Biosystems, Foster City, CA). Extended products were purified by ethanol precipitation and analyzed on an ABI 3730 automated DNA Analyzer (Applied Biosystems, Foster City, CA).

2.4. Statistical analysis

All the statistical analyses were performed with the help of SPSS statistical software (version 19.0, IBM SPSS). Power of the study was calculated using G*Power software (version 3.1). The Hardy–Weinberg equilibrium was estimated by the χ^2 test using Pypop software. For all tests, significance level was set at 5%.

3. Results

3.1. Clinical characteristics of the cohort

The clinical characteristics of PCOS cases and controls are presented in Supplementary Table 2. PCOS subjects had a significantly higher mean value for body mass index (BMI) while the mean age of menarche is significantly lower among them compared to the controls. The proportion of obese women (BMI ≥ 25) within the PCOS group was significantly higher than in the control group (55.1% vs. 15.1%, respectively; $p<0.001$). Comparison of the biochemical parameters between the lean and obese PCOS cohorts revealed that although the mean levels of LH and FSH are not significantly different between the lean and obese PCOS cases, a higher LH:FSH ratio (characteristic feature of PCOS) is evident among the obese group. Moreover, obese PCOS cases had significantly higher mean level of cholesterol and triglycerides than the lean PCOS cases (Supplementary Table 3).