CYP11A1 and CYP17 promoter polymorphisms associate with hyperandrogenemia in polycystic ovary syndrome

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Objective: To analyze promoter regions of CYP11A1 and CYP17 for putative variations in a defined group of women with polycystic ovary syndrome (PCOS) and to study their association with androgen levels.
Design: Retrospective study.
Setting: A secondary referral center for infertility at National Institute for Research in Reproductive Health, Mumbai, India.
Patient(s): One hundred women whose condition was diagnosed on the basis of the Rotterdam consensus were compared against 100 age-matched controls.
Intervention(s): A single sample of blood was collected after overnight fast on day 3 of the menstrual cycle.
Main outcome Measure(s): Plasma levels of T, androstenedione, 17α-hydroxyprogesterone, and DHEAS and nucleotide sequence of promoter regions of CYP11A1 and CYP17 genes.
Result(s): Polymorphisms in promoter regions of the two key androgen-regulating genes, CYP11A1 and CYP17, were found to be significantly associated with T levels in the cohort of well-characterized PCOS cases as compared with controls. The significance was greater in the PCOS cases with both the polymorphisms.
Conclusion(s): Our study carried out in a defined group of Indian women with PCOS suggests for the first time an individual, as well as combined, association of polymorphisms in CYP11A1 and CYP17 promoters with T levels. (Fertil Steril® 2009;92:653–9. ©2009 by American Society for Reproductive Medicine.)

Key Words: CYP11A1, CYP17, PCOS, hyperandrogenemia

Polycystic ovary syndrome (PCOS) is one of the commonest endocrinopathies affecting about 5% to 10% of women in their reproductive age. The syndrome is characterized by presence of cystic ovaries on ultrasound examination and amenorrhea and/or oligomenorrhea with clinical and/or biochemical evidence of hyperandrogenemia (1–5). Because of its extreme heterogeneous nature, the syndrome represents a range of disorders rather than a single entity (6, 7).

The pathophysiology of PCOS still has not been elucidated. However, its familial segregation, as well as evidence from linkage analysis, suggests a strong genetic component underlying its etiology (8–14). The syndrome now is suggested to be an oligogenic trait with an autosomal dominant mode of inheritance, resulting from interaction between a small number of key genes and environmental factors (15, 16). However, the underlying genetic etiology is yet to be delineated. Results of studies carried out so far in different populations have been contradictory. The major cause has been lack of consensus in definition for diagnosis of PCOS. Now a consensus definition has evolved on the basis of recommendations from an expert conference, sponsored by European Society of Human Reproduction and Embryology and the American Society for Reproductive Medicine (ESHERE/ASRM) held in Rotterdam, the Netherlands, in 2004 (17, 18).

A hallmark feature of PCOS is hyperandrogenemia with biochemical or clinical evidence of elevated androgen levels, particularly T. In previous studies carried out by us, elevated levels of T were found to be present in >30% of women with cystic ovaries on ultrasound examination, predisposing them to cardiovascular disease (19, 20). A recent study using the rhesus monkey as a model also documents that prenatal exposure to androgens produces many features characteristic to PCOS (21). Earlier a study from Nelson et al. (22) clearly showed that theca cells from PCOS ovaries produce excess of androgens both basal and in response to LH when cultured in vitro. These cells also showed overexpression of two genes, namely CYP11A1 and CYP17, which are the key regulators of androgen biosynthesis. This phenotype was retained even after several passages, indicating PCOS to be a genetic defect (22). A linkage analysis carried out by Urbanek et al. (23) with 37 candidate genes also suggested

Received January 14, 2008; revised June 23, 2008; accepted July 9, 2008; published online August 26, 2008.
M.P. has nothing to disclose. P.M. has nothing to disclose. J.G. has nothing to disclose. S.C. has nothing to disclose. A.M. has nothing to disclose.
Supported by Indian Council of Medical Research, New Delhi, India, under the program on "Genomics and Molecular Medicine" and by Lady Tata Memorial Trust (M.P.).
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some association of CYP11A1 and CYP17 with PCOS. These key genes in the androgen biosynthesis pathway therefore could serve as appropriate candidates for elucidating the genetic etiology of hyperandrogenemia associated with PCOS.

CYP11A1 encodes the enzyme P450scc, that is, cholesterol side-chain cleavage that catalyzes conversion of cholesterol to pregnenolone, an initial step in ovarian steroidogenesis. Promoter of the gene has been shown to have a pentanucleotide (tttta)n polymorphism located 528 base pairs (bp) upstream to the translation initiation site. Linkage analysis has shown strong association of this polymorphism with hyperandrogenemia and PCOS (24). Data from other researchers, however, still remains controversial.

CYP17 encodes the enzyme 17-α-hydroxylase/17–20 lyase (P45017α), which catalyzes the conversion of pregnenolone to 17-hydroxypregnenolone and P to 17-hydroxyprogesterone (17-OHP), which are rate-limiting steps in androgen biosynthesis. Increased activity of this enzyme has been postulated to contribute to enhanced androgen biosynthesis and secretion in PCOS (25, 26). Wickenheisser et al. (27) also showed that theca cells from PCOS ovaries have an increase in expression of CYP17 in vitro as compared with those from non-PCOS ovaries. A T>C polymorphism observed in the promoter region of this gene, 34 bp upstream to the translation initiation site, was found to be associated with the presence of cystic ovaries on ultrasound examination, as well as with elevated levels of androgens (28). This has been contradicted, however, by other researchers (29, 30). Data regarding both these genotypes in the Indian population are not yet available.

The present study therefore aims at screening promoter regions of CYP11A1 and CYP17 in a defined group of women with PCOS diagnosed on the basis of the Rotterdam Consensus. Because both genes encode enzymes regulating rate-limiting steps in androgen synthesis, the independent and combined association of these polymorphisms with hyperandrogenemia associated with PCOS was determined.

MATERIALS AND METHODS

The retrospective analysis was carried out in 100 women with PCOS and 100 age-matched controls. As per the consensus definition of PCOS specified in the Rotterdam Conference (2003–2004), women with evidence of any two of the following characteristics were included in the PCOS group: [1] anovulation manifested as amenorrhea and/or oligomenorrhea; [2] clinical and/or biochemical evidence of hyperandrogenemia, that is, hirsutism and/or elevated levels of plasma T; and [3] evidence of polycystic ovaries on ultrasound examination. Women with any evidence of thyroid or adrenal dysfunction or androgen-secreting neoplasm were excluded from the study.

The control group included 100 age-matched women with regular menstrual cycles, no clinical or biochemical signs of hyperandrogenemia, and normal ovaries on ultrasound examination. Women recruited in both the study groups had not received any hormone therapy for at least 3 months before inclusion.

The study was approved by the Institutional Review Board for conduct of clinical studies, and all the subjects provided informed consent before their inclusion.

A blood sample (3 mL) was collected in ethylenediaminetetraacetic acid vials from each subject. In control subjects, the samples were collected in the follicular phase, on day 3 to 6 of the menstrual cycle, whereas in subjects with PCOS, the collection was carried out on day 3 to 6 after menstruation or withdrawal bleeding.

Hormonal Estimations

Plasma from all the subjects were used for estimation of T, androstenedione (A), DHEAS, and 17-OHP by specific radioimmunoassays (Diagnostic System Laboratories, Webster, TX).

Genotyping

Genomic DNA was isolated from peripheral blood with use of a GeneElute DNA extraction kit (Sigma-Aldrich, St. Louis, MO) and genotyped for polymorphisms at promoters of CYP11A1 and CYP17.

Analysis of CYP11A1 Polymerase chain reaction (PCR) amplification of the promoter region of CYP11A1 was carried out with use of specific primers as described by Diamanti-Kandarakis et al. (31) in a Thermal Cycler (PerkinElmer, Norwalk, CT). The reaction mix of 50 μL contained 100 ng of genomic DNA, 12.5 mmol/L $MgCl_2$, 10 mmol/L deoxyribonucleoside triphosphate (dNTP) mix, in a buffer containing 500 mmol/L KCl, 100 mmol/L tris(hydroxymethyl)aminomethane (Tris) HCl (pH 8.8), 0.8 % Nonidet P 40, along with 10 pmol of each primer and 0.5 U of Taq Polymerase (MBI Fermentas, Glen Burnie, MD). Profile included initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 61°C for 1 minute, extension at 72°C for 1 minute, with a final extension step of 72°C for 10 minutes. Five microliters of the PCR product was mixed with 6× loading dye, loaded on 10% native polyacrylamide gel, and electrophoresed at 300 V for 4 hours at room temperature. The products were visualized by silver staining to study the number of pentanucleotide repeats (tttta)n, indicated by differences in sizes of the PCR products. Numbers of the repeats were confirmed further by direct sequencing with use of the 3100-Avant Genetic Analyzer and BigDye Terminator Chemistry (version 3.1; Applied Biosystems, Foster City, CA).

Analysis of CYP17 gene The 152 bp region in the promoter of CYP17 was amplified as described by Techatraisak et al. (29). Polymerase chain reaction amplification was carried out in the Thermal Cycler (PerkinElmer). Fifty microliters