Article

CYP1A1 gene polymorphism and polycystic ovary syndrome

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Abstract

The aim of this study was to assess the rates of variant alleles of cytochrome P4501A1 (*CYP1A1*) in patients with polycystic ovary syndrome (PCOS). It was designed as a case–control study in Hacettepe University, Faculty of Medicine, Department of Obstetrics and Gynecology and Genetics. Forty-eight patients with PCOS served as the study group. Ninety-six regularly cycling women with no clinical and biochemical evidence of hyperandrogenism and polycystic ovary appearance served as the controls. The *CYP1A1* variant alleles of all patients were determined via polymerase chain reaction. The rate of the *CYP1A1* isoleucine (Ile)/valine (Val) allele was significantly higher in patients with PCOS than in the controls (OR: 7.8, 95% CI: 3.45–17.52, $P < 0.001$). However, there was no statistically significant difference in the distribution of Val/Val genotype (OR: 4.0, 95% CI: 0.60–26.73). The rate of any Val genotype (Ile/Val or Val/Val) was significantly higher in patients with PCOS compared with the control group (OR: 7.4, 95% CI: 3.33–16.46, $P < 0.001$). In conclusion, the patients with PCOS had a 7.8-fold higher frequency of *CYP1A1* Ile/Val genotype and a 7.4-fold higher frequency of *CYP1A1* of any Val genotype (Ile/Val or Val/Val).

Keywords: *catechol oestrogens, CYP1A1, polycystic ovary syndrome, polymorphism*

Introduction

Polycystic ovary syndrome (PCOS) is the most common reproductive endocrinological disorder, affecting 5–10% of women of reproductive age (Franks, 1995). The aetiology of PCOS remains unclear. Genetic predisposition (Franks, 1995), altered ovarian steroidogenesis (Ehrmann *et al*., 1995; Gilling-Smith *et al*., 1997), defects in insulin signaling (Dunaif and Graf, 1989; Utiger, 1996) and neuroendocrine abnormalities (Dunaif *et al*., 1989; Utiger, 1996; Legro *et al*., 1998; Blank *et al*., 2006) have been proposed as primary aetiological factors.

There is evidence that PCOS has a genetic basis (Franks *et al*., 1997; Legro *et al*., 1998; Franks and McCarthy, 2004). A substantially increased risk of PCOS in the first-degree female relatives of PCOS patients has been reported in several studies (Hague *et al*., 1988; Carey *et al*., 1993; Norman *et al*., 1996; Legro *et al*., 1998; Govind *et al*., 1999; Kahsar-Miller *et al*., 2001). The mode of inheritance of PCOS, however, remains unclear. Recent studies revealed that PCOS represents a polygenic disorder (Franks *et al*., 1997; Diamanti-Kandarakis and Piperi, 2005; Escobar-Morreale *et al*., 2005). Many genes and their mutations and/or polymorphisms have been suggested to play a role in the pathogenesis of PCOS (Fratantonio *et al*., 2005). However, none has been unequivocally proven to play a role in the aetiology of the condition.

Oestrogens are oxidized to the 2-OH and 4-OH catechol oestrogens by cytochrome P4501A1 (*CYP1A1*) in extrahepatic tissues including the ovary (Martucci and Fishman, 1993; Yager and Liehr, 1996). Catechol oestrogens are potent inhibitors of granulosa cell replication and follicular growth, as clearly shown in the porcine model (Spicer and Hammond, 1989). *CYP1A1* has been shown to be expressed in cultured granulosa cells from developing, but not immature, follicles, indicating developmental regulation of this enzyme (Leighton *et al*., 1995). A polymorphism in the codon

462 of exon 7 (*CYP1A1*3*), which is due to an isoleucine (Ile) to valine (Val) replacement in the catalytic region, results in a change in of *CYP1A1* enzyme activity (Taioli *et al.*, 1995; Esinler *et al.*, 2006). In other words, an individual possessing *CYP1A1*3* polymorphism will have higher *CYP1A1* enzyme activity and may have inherently higher production of intraovarian catechol oestrogens. The authors postulate that, *CYP1A1* polymorphism may modulate intraovarian production of catechol oestrogens, which in turn may impair folliculogenesis and contribute to the pathophysiology of PCOS.

In this study, the rates of *CYP1A1* polymorphism have been evaluated in patients with PCOS and compared with a control group in a Turkish population.

Materials and methods

Patients and controls

The study was designed as a case–control study. The study group consisted of 48 patients diagnosed with PCOS. The diagnosis of PCOS was made by the presence of any two of the following three criteria: polycystic ovaries, oligo- or anovulation, and clinical or biochemical evidence of hyperandrogenism (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Working Group, 2004). Oligo-ovulation was defined as menstrual cycles more than 45 days in length or fewer than 8 cycles per year. Anovulation was defined as lack of ovulation for at least 3 months. Clinical hyperandrogenism was defined as the presence of acne, hirsutism (Ferriman-Gallwey score >8; Ferriman and Gallwey, 1961) or alopecia. Biochemical hyperandrogenism was defined as total testosterone greater than 81.0 ng/dl, free testosterone greater than 3.17 pg/ml, or dehydroepiandrosterone sulphate (DHEAS) greater than 430 ug/dl. Blood samples were taken on the third day of the spontaneous and/or induced menstrual cycle. Polycystic ovary appearance was characterized as the presence of 12 or more follicles in each ovary, each measuring 2–9 mm in diameter, and/or increased ovarian volume (10 ml) at transvaginal ultrasonography (Balen *et al.*, 2003). The control group constituted 96 randomly selected regularly cycling women with no clinical/biochemical hyperandrogenism and polycystic ovary (PCO) appearance. The phenotyping features of the PCOS and control groups are given in **Table 1**. All subjects in this study were of Turkish origin. Informed consent was obtained from each individual contributing to the study.

Genotyping

Blood samples from PCOS and control patients were collected and stored at –20°C. Genomic DNA was extracted from blood using the method described by Miller *et al.* (1988). The genotyping of *CYP1A1* was successfully performed for patients and control subjects using polymerase chain reaction (PCR) as described by Hayashi *et al.* (1991). Each DNA sample underwent allele-specific amplification in two separate reactions using one of two 59 primers: 59-AAG ACC TCC CAG CGG GCA AT-39 or 59-AAGACC TCC CAG CGG GCA AC-39. All reactions included the 39 primer 59-GAA AGG CTG GGT CCA CCC TCT-39 (Hayashi *et al.*, 1991). An initial denaturation at 94°C for 2 min was followed by 28 cycles at 94°C for 60 s, at 65°C for 45 s, and a final extension at 72°C for 60 s. To visualize the *CYP1A1* gene, PCR products were run on a 2% agarose gel with ethidium bromide. The PCR analysis was performed at least twice for each sample, and PCR products were identified by the presence of two bands of different sizes (Ile and Val) on the gel. Three different genotypes were defined for the individual polymorphisms, these being the homozygous wild (null) type (Ile/Ile), the heterozygous variant (Ile/Val), and the homozygous variant (Val/Val).

The error rate was very low in the control group (2.0%, 2 of 98), two samples were excluded from data and 96 control subjects were calculated. However, a zero error rate for PCOS subjects was observed.

Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences version 12.0 (SPSS Inc., Chicago, IL, USA). The chi-squared test and Fisher's exact test were used to analyse nominal variables in the form of frequency tables. Odds ratio (OR) and 95% confidence intervals (CI) were calculated from a 2 × 2 table. Student's *t*-test was used to analyse numeric values. *P*-values of 0.05 or less were considered statistically significant. Values were expressed as mean ± SD, unless otherwise stated. Cases and controls were unmatched.

Results

The incidence of *CYP1A1*3* polymorphism, in exon 7 of the gene, in the PCOS and control groups are given in **Table 2**. The rate of *CYP1A1* Ile/Val allele was significantly higher in

Table 1. Distribution of phenotypic subgroups of patients with polycystic ovary syndrome (PCOS).

Parameter	*Patients with PCOS (n = 48)*	*Controls (n = 96)*
Mean age (years) ± SD	28.5 ± 4.5	30.1 ± 6.5
Mean body mass index (kg/m^2) ± SD	27.1 ± 4.2[a]	24.5 ± 5.5[a]
No. of patients with clinical or biochemical hyperandrogenism (%)	38 (79.2)	0
No. of patients with polycystic ovaries (%)	41 (85.4)	0
No. of patients with oligo- or anovulation (%)	46 (95.8)	0

[a]$P < 0.05$.

Table 2. Distribution of *CYP1A1**3 polymorphism in control subjects (n = 96) and patients with polycystic ovary syndrome (PCOS; n = 48).

Parameter	*Ile/Ile*	*Ile/Val*	*Val/Val*	*Any Val*
Controls (%)	66 (68.7)	27 (28.1)	3 (3.1)	30 (31.2)
Patients (%)	11 (22.9)	35 (72.9)	2 (4.2)	37 (77.1)
OR[a]	1[b]	7.8	4.0	7.4
95% CI	–	3.45–17.52	0.60–26.73	3.33–16.46
Chi-squared test	–	27.57	2.32	27.01
P-value	–	<0.001	NS	<0.001

[a]OR (odds ratio) represents the relative risk for PCOS patients with Ile/Val and Val/Val genotype relative to those with Ile/Ile; [b]Reference category; NS = not statistically significant.

patients with PCOS than in the controls (OR: 7.8, 95% CI: 3.45–17.52, $P < 0.001$). However, there was no statistically significant difference in the distribution of Val/Val genotype (OR: 4.0, 95% CI: 0.60–26.73). The rate of any Val genotype (Ile/Val or Val/Val) was significantly higher in patients with PCOS compared with the control group (OR: 7.4, 95% CI: 3.33–16.46, $P < 0.001$).

Discussion

PCOS may represent a polygenic disorder (Franks *et al.*, 1997; Legro *et al.*, 1998; Franks and McCarthy, 2004). Many genes have been suggested to play a role in the pathogenesis of PCOS and the presence of their mutations and/or polymorphisms have been explored (Fratantonio *et al.*, 2005). The authors postulate that *CYP1A1* gene polymorphism may contribute to the pathogenesis of PCOS. In this study, the frequency of any Val genotype (Ile/Val or Val/Val) was significantly higher in patients with PCOS compared with regularly cycling controls (OR: 7.4, 95% CI: 3.33–16.46, $P < 0.001$).

In patients with PCOS, genes involved in steroidogenesis in theca cells, such as *CYP11a* (Gharani *et al.*, 1997), *CYP21* (Escobar-Morreale *et al.*, 1999) and *CYP17* (Carey *et al.*, 1994) have been studied. *CYP11a* codes for the P450 cytochrome side chain cleavage that converts cholesterol to pregnenolone, a rate limiting step of steroidogenesis. Gharani *et al.* (1997) found evidence for a weak linkage between the *CYP11A* 50 UTR pentanucleotide repeat polymorphism and hyperandrogenaemia in PCOS women. In another study involving *CYP11A*, an association was found between a pentanucleotide repeat at 2528 of the *CYP11A* gene and PCOS (Diamanti-Kandarakis *et al.*, 2000). However, other studies have failed to find a significant association between *CYP11A* and PCOS (Urbanek *et al.*, 1999; San Millan *et al.*, 2001).

CYP17 codes for the enzyme 17α-hydroxylase, which converts C21-steroids into androgens. Carey *et al.* (1994) reported that a rare single nucleotide polymorphism (T→C) at base pair –34 in the promoter region of this gene increases the susceptibility to develop PCOS. However, subsequent larger, case–control studies from the same group, as well as from other centres, were unable to confirm this association (Gharani *et al.*, 1996; Urbanek *et al.*, 1999). Recently, Suryanarayana *et al.* (2007) investigated the relationship between the CYP17, CYP19 polymorphisms and idiopathic recurrent miscarriages (IRM) among women in South India (143 cases and 88 controls). They noted that *CYP17* polymorphism was not associated with IRM. But they noted that *CYP19* minor genotypes (7/9, 7/10, 7/12, 8/11, 11/11, 11/12) were significantly higher in patients with IRM when compared with controls (13% versus 2%, $P = 0.006$). It was previously shown that *CYP19* is an important regulatory gene in follicular maturation, ovulation and early embryological development (Moudgal *et al.*, 1996). Escobar-Morreale *et al.* (1999) found a positive relationship between *CYP21* gene mutations and PCOS susceptibility.

The following genes have been studied in PCOS patients: genes involved in steroidogenesis in theca cells; genes involved in steroid hormone effects (androgen receptor gene, sex hormone binding globulin gene); genes involved in gonadotrophin release regulation and action (LH gene, LH receptor gene, follistatin); genes involved in insulin secretion and action (variable number tandem repeats, insulin receptor gene, insulin receptor substrate proteins IRS-1 and IRS-2, calpain 1); and genes involved in adipose tissue metabolism (leptin gene, leptin receptor gene, peroxime proliferator activated receptor) (Fratantonio *et al.*, 2005). However, genes affecting the metabolic and transport pathways of steroid hormones and accompanying molecules have not been studied. The *CYP1A1* gene is one of the most important genes affecting the metabolic and transport pathways of oestrogens.

The frequency of *CYP1A1*3* polymorphism has not been previously studied in patients with PCOS. There is only one study conducted by Babu *et al.* (2004) reporting the *CYP1A1*, glutathione *S*-transferase class μ1 (*GSTM1*) and class θ1 (*GSTT1*) genetic polymorphism in 180 women with PCO (not PCOS) and in 72 healthy fertile controls. No significant difference in the frequencies of *GSTM1* and *GSTT1* null genotypes were noted in the PCO and control groups. However, *CYP1A1 MspI* homozygous mutant was significantly more common in the PCO group (OR = 3.766; 95% CI: 1.308–10.838; $P < 0.05$).

Catechol oestrogens have been previously shown to inhibit granulosa cell DNA replication and folliculogenesis in the porcine model (Spicer and Hammond, 1989). It is here postulate that *CYP1A1* Ile/Val or any Val alleles may result in excessive production of catechol oestrogens in granulosa cells and in turn may impair replication of granulosa cells and folliculogenesis.

In conclusion, patients with PCOS have a 7.8-fold higher frequency of *CYP1A1* Ile/Val genotype and a 7.4-fold *CYP1A1* any Val genotype (Ile/Val or Val/Val). Further studies with larger sample sizes in different ethnic groups are required to delineate the association of *CYP1A1* polymorphism and pathophysiology of PCOS.

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