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CASE REPORT

Lack of an association between *CYP1A1* gene Ile462Val polymorphism and polycystic ovary syndrome in Chinese

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Abstract Polycystic ovary syndrome (PCOS) is a complex multi-factorial disorder involving a number of genetic and environmental factors. *CYP1A1* (Cytochrome P450, family 1, subfamily A, polypeptide 1) gene, which belongs to Cytochrome P450 (*CYP*) super family, encodes a phase I cytochrome P450 enzyme, involved in the oxidative metabolism of estrogens. A recent study suggested that a common polymorphism Ile462Val of the *CYP1A1* gene might be associated with PCOS development in Turkish women. To investigate a possible association between the *CYP1A1* Ile462Val polymorphism and PCOS in Chinese women, we examined 205 PCOS patients and 177 healthy controls. All subjects were genotyped for *CYP1A1*. There was no statistical difference in *CYP1A1* genotype and allele frequencies between PCOS cases and controls ($\chi^2 = 0.956$, df = 2, $P = 0.089$ by genotype; $\chi^2 = 0.005$, df = 1, $P = 0.941$ by allele). Compared with controls, there were no statistical difference in Val/Val genotype and Val allele frequency in the PCOS cases (4.9% vs. 5.1% by genotype; 51.7% vs. 52.0% by allele) ($\chi^2 = 0.009$, df = 1, $P = 0.926$ by genotype; $\chi^2 = 0.005$, df = 1, $P = 0.941$ by allele). Moreover, no association between *CYP1A1* Ile462Val genotypes and metabolic parameters was observed in PCOS women. Our findings clearly indicated that this polymorphism does not represent an additional genetic risk factor for PCOS in Chinese women.

Keywords Cytochrome P450, family 1, subfamily A, polypeptide 1 · Polycystic ovary syndrome · Polymorphism · Ile462Val · Chinese

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrinopathy and is the most common cause of infertility in women. It has been estimated that PCOS affects 4–12% premenopausal women [1].

The pathophysiology of PCOS appears to have a complex, multi-factorial etiology [2]. There is strong evidence for a genetic component in PCOS [3–5]. For example, clustering of the syndrome's phenotype is seen frequently in families of PCOS patients. To date, no study has convincingly established a mode of inheritance for the disorder [6]. Multiple genetic factors including mutations and polymorphisms have been reported to be associated with the development of PCOS [7–13]. There have been a large number of population studies attempting to discover mutations/polymorphisms that influence PCOS development using the candidate gene approach [14].

Cytochrome P450 (*CYP*) comprises a superfamily of isoenzymes that act on phase I of xenobiotic metabolic transformation. The *CYP1A1* (Cytochrome P450, family

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1, subfamily A, polypeptide 1) gene encodes a phase I cytochrome P-450 enzyme, involved in the oxidative metabolism of E2, the strong form of estrogen present in women, to the catechol estrogens 2-hydroxyestradiol (2-OHE2) in granulosa cells [15–17]. In this regard, women who carry polymorphic variants that confer higher *CYP1A1* enzyme activity may be at higher risk for PCOS [18].

One of the most common polymorphisms of CYP1A1 gene (CYP1A1*2B polymorphism (A2455G)) is Ile462-Val. There is an Ile/Val replacement at the heme-binding domain of the exon 7 region, which results in a concurrent increase in the catalytic activity of the CYP1A1 protein [19–21].

Recently, Esinler et al. [22] reported an association between the *CYP1A1* Ile/Val polymorphism and the development of PCOS in Turkish women. In the Turkish, the *CYP1A1* Ile/Val allele frequency and any Val genotypic frequency (Ile/Val or Val/Val) was higher in patients with PCOS than in controls [22]. As the authors mentioned in the article, according to the limited samples investigated, further studies with larger sample sizes in different ethnic groups are required to verify their findings.

To investigate a possible association between the *CYP1A1* Ile/Val polymorphism and PCOS in Chinese, we examined *CYP1A1* genotypes in PCOS patients and controls from China.

Materials and methods

Subjects

A total of 382 unrelated Chinese women were recruited from the First Affiliated Hospital, Anhui Medical University. A total of 205 patients with PCOS and 177 were healthy controls. The diagnosis of PCOS was based on the criteria of Rotterdam Revised 2003 (2 out of 3) diagnosis: oligomenorrhea or amenorrhea for at least 6 months; clinical and/or biochemical signs of hyperandrogenism; polycystic ovaries (presence of 12 or more follicles in each ovary measuring 2–9 mm in diameter, and/or increased volume (10 ml). Congenital adrenal hyperplasia, Cushing's syndrome, androgen-secreting tumor, hyperprolactinemia, and thyroid dysfunction were excluded. Control subjects of proven fertility, with normal menstrual cycles and ovary morphology, and without a history of subfertility treatment, were recruited from the Anhui Medical University. The study was approved by the Ethics Committee of the National Research Institute for Family Planning and informed consent was obtained from all participants.

Biochemical and hormonal measures

Blood samples were drawn on the third day of menstrual onset in the early follicular phase of the cycle after an overnight fast. Plasma was harvested and stored at −20°C until total testosterone, prolactin (PRL), follicle stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2) were determined.

DNA analysis

Blood samples from PCOS patients and controls were collected and stored at −20°C. Genomic DNA was extracted from peripheral blood leukocytes using standard methods [23]. The genotyping of *CYP1A1* was performed for patients and control subjects using allele-specific polymerase chain reaction (AS-PCR). Each DNA sample underwent allele-specific amplification in two separate reactions using three primers: one reaction used the 5-primer: 5′-AAG ACC TCC CAG CGG GCA AT-3′ and the 3-primer: 5′-GAA AGG CTG GOT CCA CCC TCT-3′. For the other reaction we chose the 5-primer: 5′-AAG ACC TCC CAG CGG GCA AC-3′ and the 3-primer: 5′-GAA AGG CTG GOT CCA CCC TCT-3′. An initial denaturation at 94°C for 3 min was followed by 45 cycles at 94°C for 60 s, at 64°C for 60 s, and a final extension at 72°C for 60 s. Two samples with foregone different homozygous genotypes according to sequencing results were used as false positive controls in each PCR. To visualize the *CYP1A1* gene, PCR products were run on a 3% agarose gel with ethidium bromide. The PCR analysis was performed at least twice for each sample, and two kinds of PCR products of each sample were identified by the presence of two bands of same sizes (209 bp) on these gels. Three different genotypes were defined, the homozygous wild type (Ile/Ile), the heterozygous variant (Ile/Val), and the homozygous variant (Val/Val).

Statistical analysis

In this study, statistical analyses were carried out using the Statistical Package for Social Sciences version 10.0 (SPSS 10.0). Differences between noncontiguous variables, genotype distribution and allele frequency, were tested by chi-square analysis. Student's *t*-test was used to compare data of the clinical parameters (age at menarche, age at menarche, E2 levels, FSH levels, LH levels, PRL levels, and total testosterone levels) between different genotypes. Significant differences between or among groups was indicated by a P-value < 0.05.

Results

Clinical and laboratory variables

The clinical characteristics (mean + SD) of women enrolled in the study are summarized in Table 1. There were no significant differences between PCOS women and controls when comparing their serum FSH and PRL levels. The E2 and LH levels in PCOS group were significantly higher than controls. In addition, PCOS women presented a younger mean age and a significantly higher BMI values when compared with controls.

Genotype and allele frequencies

The distribution of the different *CYP1A1* genotypes in the study population is shown in Table 2.

There was no statistical difference in *CYP1A1* genotype and allele frequencies between PCOS cases and controls.

Compared with controls, there was a higher Val/Val genotype and Val allele frequency in the PCOS cases (4.9% vs. 5.1% by genotype; 51.7% vs. 52.0% by allele), but the association with PCOS did not reach significance.

In addition, the clinical/biochemical parameters were investigated among the different groups of genotypes, including the mean ages of menarche, BMI values, E2 levels, FSH levels, LH levels, PRL levels, and total testosterone levels to determine if there was a possible association between *CYP1A1* gene polymorphism and clinical/biochemical parameters in women of the PCOS group (shown in Table 3). But all these associations to clinical/biochemical parameters did not reach significance ($P > 0.05$).

Discussion

Multiple genetic pathways have been implicated in the pathogenesis of PCOS including steroid hormone

Table 1 Demographic and clinical characteristics (mean ± SD) of the study population

Parameter	PCOS ($n = 205$)	Control ($n = 177$)	P
Age (year)	26.84 ± 4.05	31.14 ± 4.22	$P = 0.650$
Menarche age (year)	14.43 ± 2.06	14.33 ± 1.38	$P = 0.567$
BMI (kg/m^2)	23.16 ± 4.17	21.37 ± 2.47	$P < 0.001$
E2 levels (pg/ml)	233.1 ± 208.0	176.1 ± 124.6	$P = 0.001$
FSH levels (mIU/ml)	5.46 ± 2.45	6.71 ± 2.13	$P = 0.35$
LH levels (mIU/ml)	13.07 ± 7.00	4.98 ± 3.12	$P < 0.001$
Prolactin levels (ng/ml)	17.57 ± 32.93	18.07 ± 19.46	$P = 0.85$
Total testosterone (ng/ml)	3.45 ± 8.45	2.07 ± 6.96	$P = 0.081$

BMI body mass index; *E2* estradiol; *FSH* follicle-stimulating hormone; *LH* luteinizing hormone

Table 2 Genotype distribution and relative allele frequencies of Ile462Val polymorphism of *CYP1A1* gene in Chinese with PCOS ($n = 205$) and controls ($n = 177$)

Group	No.	Genotype frequency (%)			Allele frequency (%)	
		I/I	I/V	V/V	I	V
PCOS	205	3(1.4)	192(93.7)	10(4.9)	198(48.3)	212(51.7)
Controls	177	2(1.1)	166(93.8)	9(5.1)	170(48.0)	184(52.0)
		$\chi^2 = 0.956$, df = 2, $P = 0.089$			$\chi^2 = 0.005$, df = 1, $P = 0.941$	

Table 3 Biochemical profile (mean ± SD) of Chinese PCOS women according to genotypes for Ile462Val polymorphism of *CYP1A1* gene

Parameter group	I/I + I/V ($n = 195$; 95.1%)	V/V ($n = 10$; 4.9%)	P
Menarche age	14.45 ± 2.08	14.00 ± 1.63	$P = 0.5$
BMI	23.22 ± 4.22	22.12 ± 3.10	$P = 0.416$
E2 levels (pg/ml)	234.74 ± 211.83	200.0 ± 109.97	$P = 0.617$
FSH levels (mIU/ml)	5.43 ± 2.49	5.95 ± 1.46	$P = 0.512$
LH levels (mIU/ml)	13.23 ± 7.02	9.94 ± 6.05	$P = 0.147$
Prolactin levels (ng/ml)	17.79 ± 33.74	13.32 ± 5.69	$P = 0.677$
Total testosterone (ng/ml)	3.48 ± 8.66	2.86 ± 1.35	$P = 0.823$
LH/FSH	2.92 ± 3.29	1.73 ± 0.97	$P = 0.256$

metabolism, gonadotropin action, obesity and energy regulation, and insulin action [24].

Increasing evidence from biological functional and genetic studies indicate that the *CYP1A1* gene may play a role in PCOS development [25, 26]. Therefore, the study of genetic polymorphisms related to enzyme involved in the metabolism of estrogen metabolism, combined with research regarding known environmental risk factors, may provide important information to evaluate the risk of development of PCOS.

The results of Ibrahim's study among Turkish women (48 PCOS cases plus 96 controls) suggested that CYP1A1 Ile/Val polymorphism presented a risk factor in PCOS developing. The significant differences of both genotypic and allelic frequencies were found between Turkish PCOS patients and controls.

In the present study, we have investigated the possible association between the Ile/Val polymorphism at the *CYP1A1* gene and PCOS in more than twice as many Chinese women. Our observations show no difference in genotype and allele distribution in these women compared to that of the normal Chinese women who participated in this study. Our observations strongly suggest that this polymorphism is not associated with PCOS in Chinese.

There are several difference observed in the results of these two studies.

First, it should be noted that our study has more than sufficient statistical power to have replicated the findings with Turkish women [22], if such a relationship existed in the Chinese population used in our study. In contrast, the study with Turkish women was performed using a much smaller sample of affected (48 cases) and unaffected (96 controls) individuals. It is possible that the latter situation may have resulted in a statistically false positive effect.

Secondly, a certain genetic variant may interact with other variants and local environmental influences such that it alters phenotype only in a particular group. Still, it should be noticed that the frequency of Ile/Val genotype in present study is much higher than that in Ibrahim's study (93.8% vs. 28.1%), which suggested an obvious ethnic difference. This may be another possible reason for the difference in the results of the previous study and our present study, which uses a population of Chinese women (For example, in Hao's study [27]).

Third, the frequency of the Ile/Val genotype in our study appears to be higher than that in the Turkish study [22]. This may indicate an ethnic difference between the two populations, which may explain, in part, the divergent observation between this earlier study and our present results.

In conclusion, the dichotomy between our observation with Chinese women and those obtained with Turkish women emphasized the need for further research involving large groups of women in different populations to clarify the role of the *CYP1A1* gene Ile462Val polymorphism in the pathogenesis of PCOS in specific ethnic populations.

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