ORIGINAL ARTICLE

Evaluation of association between the CYP11α promoter pentannucleotide (TTTTA)n polymorphism and polycystic ovarian syndrome among Han Chinese women

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Abstract

Evidence indicates that CYP11α is a positional and functional candidate for genetic study in polycystic ovarian syndrome (PCOS). In the present study, we have evaluated the association between the *CYP11α* promoter pentannucleotide (tttta)n polymorphism and PCOS among Han Chinese women. Subjects include 125 patients with PCOS and 121 healthy controls, and all were Han Chinese women. Clinical characteristics of patients with PCOS and control subjects were examined according to the Rotterdam consensus criteria. The *CYP11α* promoter pentannucleotide (tttta)n polymorphism was genotyped with PCR and fluorescent capillary electrophoresis protocol. Results indicate that common alleles of the CYP11α promoter pentannucleotide (tttta)n polymorphism in this population of Han Chinese women were P4, P6 and P8, and allele P6 was the most common one. Frequencies of those three common alleles between PCOS cases and controls (24.8, 65.6, 6.8% and 23.6, 65.7, 9.9%, respectively) were similar. No significant allelic association of this polymorphism with PCOS was found. However, the carriers with allele P6 among patients with PCOS had increased WHR (0.85±0.05 vs 0.82±0.08, p=0.039) and decreased AUCG (9.5±2.1 vs 11.4±2.8, p=0.021) compared to the patients carrying other alleles. Therefore, the most common allele of the *CYP11α* promoter pentannucleotide (tttta)n polymorphism in the population of Han Chinese women is P6, while the most common allele in European Caucasians, as previously reported, is P4. This polymorphism is an ethnic and racial variant, and may have the risk susceptibility in abnormal metabolism of patients with PCOS in Han Chinese women.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women, which is influenced by genetic and environmental factors (2,5). In China, PCOS effects 50–60% of outpatients in gynecologic endocrinopathy clinics among Han Chinese women at reproductive age (12). Clinical and biochemical evidence indicates that PCOS is a heterogenous endocrine disorder associated with amenorrhoea, hyperandrogenism, hirsutism, obesity, insulin resistance and an approximately 7-fold increased risk of type 2 diabetes (4,11,12). There is also strong evidence for a major genetic component in the aetiology of PCOS. Familial aggregation of PCOS has been well established (2,5,12,14). Thus, PCOS is a multi-factorial disease. Identification of the susceptibility gene and its polymorphism(s) may provide useful information for a better understanding of its pathogenesis.

The gene of cytochrome P450, family 11, subfamily A, polypeptide 1 (GeneID: 1583) is encoded as *CYP11α*. The alternative names for this gene are *CYPXIA1, P450scc* and *P450*. CYP11α catalyzes the side-chain cleavage reaction of cholesterol to pregnenolone. Biological evidence indicates that androgen production and CYP11α expression were increased in thecal cells cultured from PCOS patients in comparison with samples from control women (8,10). The *CYP11α* gene is located on chromosome 15q24.1 (1), where is linked to PCOS (3,6). Therefore, CYP11α has been considered as a functional and positional candidate gene for genetic study in PCOS.

In the *CYP11α* gene, there resides a pentanucleotide (tttta)n repeat polymorphism (D15S520) at position –528 from the start of translation site in the promoter region. In the recent years, several genetic association studies have been carried out. The data have demonstrated that this polymorphism is moderately associated with PCOS in Greece women but not in Hirsute women (3). In the population of UK women, although the allelic association of this polymorphism with PCOS was detected, no discernable relationship between genotypes of this polymorphism and androgen-related phenotypes was found (7). Last year, a case and control association study has indicated that P6 is the most common allele of this polymorphism in a population of Han Chinese women, while this allele is weakly associated with BMI in the patients with PCOS (16). In the present study, we have carried out a genetic association study with PCOS patients selected from another population of Han Chinese women. The aims are to further evaluate the association between the polymorphism and PCOS among Han Chinese women and also to ascertain whether the *CYP11α* pentanucleotide polymorphism is an ethnic and racial variant.

MATERIAL AND METHODS

Subjects

A total of Chinese women, including 125 patients with PCOS and 121 healthy controls, were included in the present study. All of them had Han Chinese nationality and were selected from Shangdong province, China. The patients with PCOS were diagnosed based on the presence of two out of three criteria of Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group, including oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries. Other aetiologies (congenital adrenal hyperplasias, androgen-secreting tumours and Cushing's syndrome) were excluded. All participants gave their informed consent to take part in the study. Procedures followed were in accordance with the ethical standards of the responsible committee of human experimentation. Clinical characteristics of the patients with PCOS and healthy control subjects are summarized in Table 1.

Table 1. Clinical and endocrine characteristics in Han Chinese women with and without PCOS

	PCOS cases	Controls	P-value
N	125	121	
Age (yrs)	30.9±7.9	30.3±6.4	0.749
BMI (kg/m^2)	25.3±4.0	22.7±2.1	0.017
WHR	0.82±0.06	0.77±0.05	0.002
SBP (mmHg)	119±10	112±10	0.008
DBP (mmHg)	80±9	73±9	0.007
FSH (IU/L)	6.67±2.20	7.02±1.97	0.517
LH (IU/L)	11.35±6.72	5.66±2.93	<0.001
PRL (ng/ml)	13.60±9.53	14.75±7.86	0.261
E2 (pg/ml)	46.56±27.90	37.46±17.38	0.253
T (ng/ml)	62.59±23.95	51.57±22.62	0.003
SHBG (mmol/L)	151.27±60.83	194.31±75.24	0.002
FAI	1.72±0.87	0.83±0.55	0.002
IR	1.71±0.98	0.81±0.41	0.004
AFC	11.34±4.77	6.12±1.87	<0.001
TG (mmol/L)	0.97±0.71	0.67±0.38	0.035
CHO (mmol/L)	5.53±0.72	5.07±0.79	0.087
LDL-C (mmol/L)	4.74±0.72	4.13±0.81	0.247
HDL-C (mmol/L)	1.13±0.20	1.41±0.29	0.005
AUCG	11.02.±1.69	6.46±1.31	0.005
AUCI	57.54±23.77	37.99±16.42	0.018

Data are means ± SD.

Table 2. Allele and genotype frequencies of the CYP11α (tttta)n polymorphism in Han Chinese women with and without PCOS

Locus	Alleles/Genotypes	PCOS cases N (%)	Controls N (%)	*p*-value
Genotype counts	P4/P4	8 (6.4)	8 (6.6)	0.946
	P4/P6	38 (30.4)	34 (28.1)	0.692
	P4/P8	4 (3.2)	6 (5.0)	0.485
	P6/P6	57 (45.6)	55 (45.5)	0.982
	P6/P8	12 (9.6)	14 (11.6)	0.615
	Others*	5 (4.0)	4 (3.3)	
Allele counts	P4 (169 bp)	62 (24.8)	57 (23.6)	0.747
	P6 (179 bp)	164 (65.6)	159 (65.7)	0.981
	P8 (189 bp)	17 (6.8)	24 (9.9)	0.211

*Genotypes with less 3% frequencies included P4/P7, P4/P9, P4/P10, P6/P10, P8/P8 and P8/P9. The rare alleles P7, P9 and P10 were not included in the comparison tests.

Methods

Peripheral blood samples were collected on days 2–5 of spontaneous cycle or after withdrawal of bleeding with the subjects in a fasting state. The blood samples were collected at 0, 30, 60, 120 and 180 min of the 75g oral glucose tolerance test (OGTT) in all patients with PCOS. Measurement of hormones, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL) and testosterone (T) was done with a chemiluminescent analyzer (Beckman Access Health, USA). Serum levels of sex hormone-binding globulin (SHBG) were measured by using an immunoradiometric assay kit (DSL corporation, USA). Serum glucose and insulin levels were examined by enzymatic and chemiluminescent methods, respectively. The free androgen index (FAI) was calculated according to the formula of T (nmol/L) × 100/SHBG (nmol/L). Additionally, body mass index (BMI kg/m^2) was calculated according to the World Health Organization (WHO) criteria.

Genomic DNA was isolated from peripheral blood samples using a DNA purification kit (Tiangen Biotech Co., China). As the same as in the previous study, the sequences PCR primers directly labeled with fluorescence were 5'-GGT GAA ACT GTG CCA TTG C-3' (forward) and 5'-GTT TGG GGG AAA TGA GGG GC-3' (reverse) (Bioengineering Co. Shanghai). PCR experiments were performed by using a Hot Start PCR protocol and instrument of ABI 9700 (Applied Biosystems, USA). To detect the genotypes of the *CYP11α* pentanucleotide polymorphism, analysis of capillary electrophoresis with ABI Prism 3100-Avant hereditary analyzer (Applied Biosystems, USA) and software of Gene Scan 3.7 (Applied Biosystems, USA) was used according to the operative illustration. The glucose and insulin responses to the OGTT were analysed by calculating the area under the curve (AUCG, area under the curve of glucose).

Statistical analyses

Genotype and allele distribution between cases (women with PCOS) and healthy controls was compared using the Pearson χ^2 test. Differences in clinical and metabolic variables between individuals with different genotypes were tested by using Student's *t* tests or ANOVA. *p*-values less than 0.05 were interpreted as statistically significant. All statistical analyses were performed with SPSS statistical package, version 11.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

We have carried out a genetic association study for the *CYP11α* promoter pentanucleotide polymorphism with the clinical material of 125 Chinese women with PCOS and 121 healthy controls. Genotype distributions and allele frequencies in cases and controls are presented in Table 2. Among the patients with PCOS, the carriers with genotypes P6/P6, P4/P6 and P6/P8 were 57 (45.6%), 38 (30.4%) and 12 (9.6%), respectively, while the carriers with these three different genotypes in controls were 55 (45.5%), 34 (28.1%) and 14 (11.6%). Obviously, there were three common alleles (at least 3%), including P4 (169 bp), P6 (179 bp) and P8 (189 bp), and P6 is the most common allele (65.6–65.7%) in this population of Han Chinese women. The frequencies of alleles P4, P6 and P8 between cases and controls were similar and no significant difference was found (24.8% vs 23.6% p=0.981; 65.6% vs 65.7% p=0.747; 6.8% vs 9.9% p=0.211).

We have attempted detect the interaction between genotypes and phenotypes, although no significant allelic association was found. Analyses for quantitative traits among the carriers with each allele P4, P6 and P8 were done. We found that the carriers with allele P6 among the patients with PCOS had similar BMI (25.3±4.5 vs 25.6±2.8 kg/m^2, p=0.571) but increased WHR (0.85±0.05 vs 0.82±0.08, p=0.039) and decreased

AUCG (9.5±2.1 vs 11.4±2.8, p=0.021) (Figure 1). No statistically significant association with other clinical features in either the patients with PCOS or in healthy control subjects was detected (Data not shown).

In order to ascertain whether the *CYP11α* promoter pentanucleotide polymorphism is an ethnic and racial variant, we have summarized the allele frequencies of the patients with PCOS and healthy controls in three cohorts of European Caucasians and two cohorts of Han Chinese populations from the previous and present studies (Table 3). In European Caucasian populations, including Greek, Hirsute and UK women, P4 is the most common allele with the frequencies from 51.5% to 67.6%, while P6 allele is the second common allele with the frequencies from 17.6% to 36.1%. Among Han Chinese women, however, the frequency of P4 allele (from 17.4% to 24.8%) is less than P6 allele (from 65.6% to 74.9%).

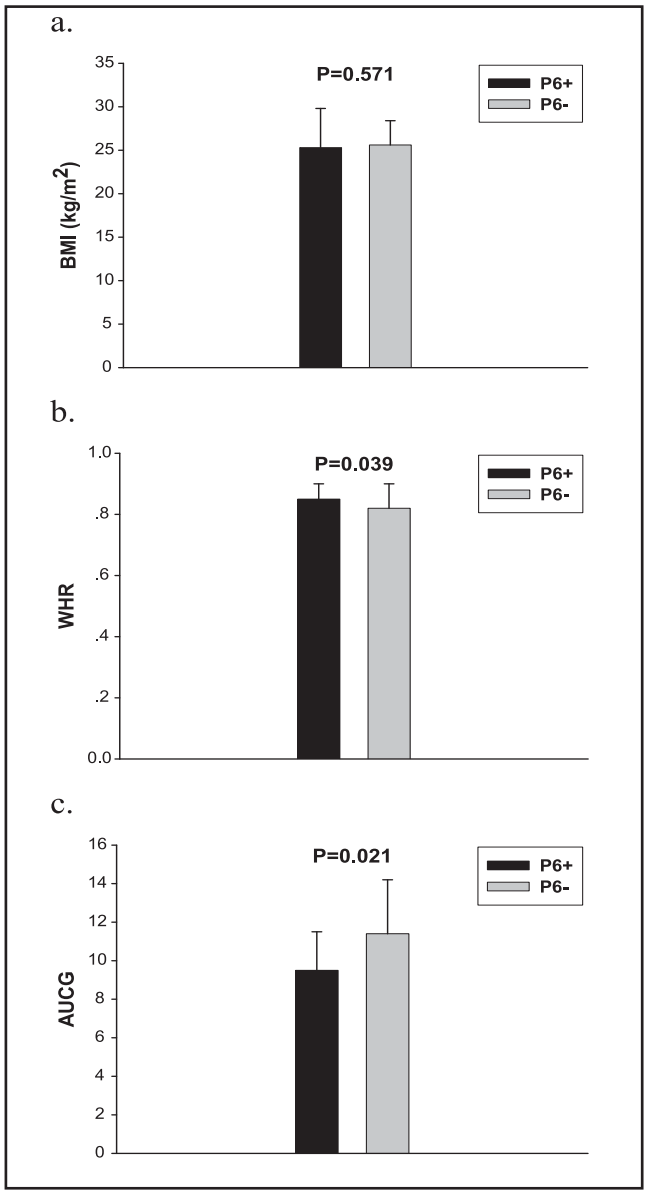

Figure 1. Comparison analyses between the PCOS patients carrying with and without allele P6 in the *CYP11α* promoter pentannucleotide (tttta)n polymorphism. There was no significant difference in BMI (a) between the carriers with and without allele P6. But, this allele was found to be significantly associated with increased WHR (b) and decreased AUCG (c).

DISCUSSION

Polycystic ovary syndrome (PCOS) is one of the most common endocrine and metabolic disorders of reproductive-aged women. It is estimated that the prevalence of this disease is 5%–10% .The influence of PCOS refers to a great many aspects, not only resulting in infertility. Firstly, most of patients usually have endocrine disorders, such as hyperandrogenism and high serum luteining hormone (especially for women with PCOS who have normal body mass index). Secondly, to a large extent, women with PCOS are inclined to have disturbance of lipid metabolism and abnormality of blood pressure. Along with this trait, Insulin resistance is a usual phenomenon, accompanying with PCOS. All in all, PCOS is a multisystem disease leading to plenty of exceptional states. In our study, the analysis of relevant parameters, which are to evaluate the states of endocrine and metabolic disorders, are in accordance with the manifestations of polycystic ovary syndrome. What is more, the consequences also provide persuasive evidences for the inclusion of PCOS.

We have carried out the genetic association study of the *CYP11α* promoter pentanucleotide polymorphism in Han Chinese women with PCOS. There is no allelic association of this polymorphism with PCOS in this population of Han Chinese women. But, we found that the allele P6 is significantly associated with increased WHR and decreased AUCG (area under the curve of glucose) in the patients with PCOS. Recently, a report in another population of Han Chinese women has demonstrated that the allele P6 is weakly associated with BMI in the patients with PCOS, and our study have analogous conclusions. In the study, we found that no significant difference existed in BMI between women with allele P6 and women without allele P6, and yet, the analysis about the parameter of WHR had discrepancy. Patients with alleles P6 had higher WHR than patients without allele P6. Therefore, we concluded that WHR might be a better index to evaluate the concentric obesity. In addition, for the first time, our study demonstrated that carriers with allele P6 had lower AUCG (area under the curve of glucose), compared to patients without allele P6. This provides us a revelation, namely, whether allele P6 is a protective gene to relieve the severity of impaired glucose tolerance, which needs further study. The genotyping methods used in that study are PCR and conventional polyacrylamide gel electrophoresis (16). In the present study, we have performed the genotyping experiments with advanced fluorescent capillary electrophoresis protocol. Although the methods used for genotyping experiments in that report and our present study are different, results from both studies are consist-

ent each other, and suggest that P6 is the most common allele in Han Chinese women. This allele may confer the genetic influence to the metabolic features in the development of PCOS in Han Chinese women. Genetic variation in the promoter may alter the sequences for gene regulation and expression (1). Therefore, it may be necessary to further investigate the biological role of the specific alleles of this polymorphism in term of the *CYP11α* promoter activity.

Interestingly, we have confirmed that P6 is the most common allele of the *CYP11α* promoter pentanucleotide polymorphism in the population of Han Chinese women. The previous studies have demonstrated that P6 is not the most common allele in European Caucasians. Instead, the allele P4 has the highest frequencies (3,7,13). Taking the previous and present study together, the patterns of genotype distributions in the studied populations of European Caucasians and Han Chinese predict that this polymorphism is an ethnic and racial variant.

In conclusion, the present study provides the further evidence that the most common allele of the *CYP11α* promoter pentannucleotide (tttta)n polymorphism in the population of Han Chinese women is P6. This allele may confer the susceptibility in abnormal metabolism of patients with PCOS. Furthermore, comparing with the previous studies in European Caucasians, we suggest that this polymorphism is an ethnic and racial variant, which differently distributes the genotypes between European Caucasians and Han Chinese women populations.

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