ORIGINAL ARTICLE

Evaluation of the association between the CYP19 Tetranucleotide (TTTA) n Polymorphism and Polycystic Ovarian Syndrome(PCOS) in Han Chinese Women

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Abstract

OBJECTIVE: Evidence indicates that the CYP19 gene is a positional and functional candidate for genetic study in polycystic ovarian syndrome (PCOS). The present study aims to evaluate the association between tetranucleotide TTTA repeat polymorphism in the CYP19 gene and PCOS among Han Chinese women.

METHODS: Clinical materials employed in this study consist of 123 patients with PCOS and 113 healthy controls. The CYP19 tetranucleotide TTTA repeat polymorphism was genotyped with a protocol of PCR and fluorescent capillary electrophoresis.

RESULTS: Common allele of the CYP19 tetranucleotide TTTA repeat polymorphism in this population of Han Chinese women was 11R. The frequency of 11R in PCOS was lower than in the control subjects (34.55% vs 42.92%, p=0.046). The carriers with allele 11R in PCOS had decreased CHO (5.00±0.63 vs 6.14±0.85 mmol/L, p=0.012). The carriers with allele 7R-TCT in PCOS had increased CHO (5.96±0.83 vs 5.08±0.65 mmol/L, p=0.027) and LDL (5.11±0.77 vs 4.31±0.66 mmol/L, p=0.014) compared to the patients carrying other alleles.

CONCLUSIONS: The most common allele of the tetranucleotide TTTA repeat polymorphism in the forth intron of CYP19 gene in Han Chinese women is 11R, which was different with the previous study in European Caucasians. Allele 11R may be associated with PCOS in the population of Han Chinese women, and it may refrain from the hypercholesteremia of PCOS. Allele 7R-TCT may be related to the lipid metabolism of PCOS. This CYP19 tetranucleotide TTTA repeat polymorphism is an ethnic and racial variant and moderately contributes to the pathogenesis of PCOS in the population of Han Chinese women.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women, which is influenced by genetic and environmental factors (Diamanti-Kandarakis *et al.* 2006; Franks *et al.* 2006). In China, PCOS effects 50–60% of outpatients in gynecologic endocrinopathy clinics among Han Chinese women at reproductive age (Su 2001). Clinical and biochemical evidence indicates that PCOS is a heterogenous endocrine disorder associated with amenorrhoea, hyperandrogenism, hirsutism, obesity, insulin resistance and predispose to type 2 diabetes and atherosclerosis (Ovalle & Azziz 2002; Christian *et al.* 2003; Legro 2003). There is also strong evidence for a major genetic component in the aetiology of PCOS (Fratantonio 2005). Familial aggregation of PCOS has been well established (Diamanti-Kandarakis *et al.* 2006; Franks *et al.* 2006; Su 2001). Thus, PCOS is a multi-factorial disease. Identification of the susceptibility genes and their polymorphism(s) may offer useful understanding of molecular mechanisms underlying pathogenesis of PCOS.

Cytochrome P450 aromatose is the key enzyme to catalyze C19 cholesterol (androgen) to C18 cholesterol (estrogen) and play an important role in estrogen biosynthesis (Simpson *et al.* 1994; Chen *et al.* 1988). P450arom is present in the endoplasmic reticulum of cells in which it is expressed, including granulosa cells and corpus luteum of the ovary, the Leydig cells of the testis, placenta, various sites in brain and in adipose tissue. Biological evidence indicates that P450arom mRNA expression in the granulosa cells of PCOS patients remained low (Ito *et al.* 1993). This indicates that estrogen production is low in PCOS follicles because there is insufficient aromatase stimulating bioactivity to increase P450arom mRNA expression. Reduced aromatase activity may lead the development of PCOS, since PCOS has been observed in the patients with aromatase deficiency caused by rare loss-of-function mutations (Ito *et al.* 1993). The cytochrome P450arom is encoded by CYP19 gene (GeneID: 1588), which is located on chromosome 15q21.2 and comprised of a 30 kb coding region and a 93 kb regulatory region (Simpson *et al.* 1994). CYP19 has been considered as a functional and positional candidate gene for genetic study in PCOS. Therefore, it is necessary to investigate whether the genetic influence of CYP19 gene plays a role in the pathogenesis of PCOS in Han Chinese women.

In the present study, we have carried out a genetic association study with PCOS patients in the 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 tetranucleotide TTTA repeat polymorphism in the forth intron of CYP19 gene is an ethnic and racial variant.

MATERIAL AND METHODS

Subjects

A total of 236 Han Chinese women, including 123 patients with PCOS and 113 healthy controls, were included in the present study. The patients with PCOS and the healthy control subjects were diagnosed and examined at the reproductive medical center of Shandong Provincial Hospital, Shandong University, China. The healthy control subjects were the infertile patients because of the fallopian tube or the male factors. 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.

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 75 g oral glucose tolerance test (OGTT) in all patients with PCOS. Measurement of hormones, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), test-osterone (T) and insulin was done with a chemiluminescent analyzer (Beckman Coulter, Inc. Fullerton, CA, USA). Serum levels of sex hormone-binding globulin (SHBG) were measured by using an immunoradiometric assay kit (DSL, Inc. Webster, TX, USA). Serum glucose, triglyceride (TG), cholesterol (CHO), low-density lipoprotein(LDL) and High-density lipoprotein(HDL) were measured by enzymatic and chemilunescent methods (OLYMPUS AU2700, JAPAN). The free androgen index (FAI) was calculated according to the formula of T (nmol/L) × 100/SHBG (nmol/L). Body mass index (BMI kg/m^2) was calculated according to the World Health Organization (WHO) criteria. The glucose and insulin responses to the OGTT were analysed by calculating the area under the curve (AUC).

Genomic DNA was isolated from peripheral blood samples using a DNA purification kit (Tiangen Biotech Co., Ltd. Beijing, China). As the same as in the previous study, the sequences of PCR primers labeled with fluorescence were 5`-GCA GGT ACT TAG TTA GCT AC-3` (forward) and 5`-TTA CAG TGA GCC AAG GTC GT-3` (reverse) (Sangon Biotech Co., Ltd. Shanghai, China). 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 CYP19 tetranucleotide TTTA repeat 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.

Statistical analyses

Genotype and allele distribution between patients 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 CYP19 tetranucleotide TTTA repeat polymorphism in 123 Chinese women with PCOS and 113 healthy controls. Genotype distributions and allele frequencies in cases and controls are presented in Table 2. We found 6 alleles of the CYP19 tetranucleotide TTTA repeat polymorphism including 7R-TCT, 7R+TCT, 11R,12R,8R and 10R in this population of Han Chinese women. The common alleles were 7R-TCT, 7R+TCT, 11R and 12R, and their frequencies were 30.08, 27.24, 34.55, 7.32% in the PCOS cases and 24.34, 24.78, 42.92, 5.75% in the control subjects. The frequency of 11R in the patients with PCOS was lower than in the controls and the *p*-value was the borderline statistically significant (34.55% vs 42.92%, p=0.046). The frequencies of alleles 7R-TCT, 7R+TCT and 12R between cases and controls were similar and no significant difference was found (p=0.191, 0.605, 0.277). Allele 8R was only found in the patients with PCOS, while the 13R was only found in the control group. Allele 9R was not found in the studued population.

We performed an analysis for single-marker association between the CYP19 tetranucleotide TTTA repeat polymorphism and quantitative traits of patients with PCOS. We found that the carriers with allele 11R among the patients with PCOS had decreased CHO (5.00±0.63 vs 6.14±0.85 mmol/L, p=0.012) and the carriers with allele 7R-TCT among the patients with PCOS had increased CHO (5.96±0.83 vs 5.08±0.65 mmol/L, p=0.027) and LDL (5.11±0.77 vs 4.31±0.66 mmol/L, p=0.014) compared to the patients carrying other alleles (Figure 1). No statistically significant association with other clinical features either in the patients with PCOS or in healthy control subjects was detected (Data not shown).

Tab. 1. Clinical and endocrine characteristics of the patients with PCOS and healthy control subjects.

	PCOS cases	Controls	*p*-value
N	123	113	
Age (yrs)	30.9±7.5	30.7±6.5	0.749
BMI (kg/m^2)	25.3±3.8	22.8±2.1	0.018
WHR	0.82±0.06	0.76±0.05	0.002
SBP (mmHg)	119±9.9	110.9±10	0.041
DBP (mmHg)	80±9	72±9.1	0.039
FSH (IU/L)	6.66±2.17	6.98±1.59	0.517
LH (IU/L)	11.65±6.09	5.87±3.03	0.008
T (ng/mL)	63.04±24.02	50.79±22.95	0.003
SHBG (mmol/L)	151.48±60.77	190.71±73.83	0.002
FAI	1.71±0.86	0.82±0.60	0.002
HOMA-IR	1.70±0.97	0.81±0.52	0.002
TG (mmol/L)	0.97±0.69	0.67±0.42	0.035
CHO (mmol/L)	5.52±0.74	5.09±0.77	0.087
LDL (mmol/L)	4.72±0.68	4.24±0.83	0.247
HDL (mmol/L)	1.12±0.21	1.41±0.30	0.005
AUC glucose	11.31±1.71	6.50±2.07	0.005
AUC insulin	57.74±24.87	38.01±16.79	0.018

Data are means±SD.

Tab. 2. Allele and genotype frequencies of the CYP19 (tttta)n polymorphism in the patients with PCOS and healthy control women.

Locus	Alleles/ Genotypes	PCOS cases N (%)	Controls N (%)	p-value
Genotype counts	7R+TCT/11R	23 (18.70)	13 (11.50)	0.125
	7R-TCT/11R	22 (17.89)	23 (20.35)	0.630
	7R-TCT/7R+TCT	22 (17.89)	13 (11.50)	0.168
	11R/11R	17 (13.82)	26 (23.01)	0.068
	7R-TCT/7R-TCT	12 (9.76)	7 (6.19)	0.315
	7R+TCT/7R+TCT	8 (6.50)	13 (11.50)	0.178
	7R+TCT/12R	6 (4.88)	3 (2.65)	0.582
	7R-TCT/12R	5 (4.07)	5 (4.42)	1.000
	11R/12R	5 (4.07)	5 (4.42)	1.000
	Others*	4 (3.25)	5 (4.42)	0.897
Allele counts	7R-TCT (168bp)	74 (30.08)	55 (24.34)	0.191
	7R+TCT (171bp)	67 (27.24)	56 (24.78)	0.605
	11R (187bp)	85 (34.55)	97 (42.92)	0.046
	12R (191bp)	18 (7.32)	13 (5.75)	0.277

*Genotypes with less 3% frequencies included 7R-TCT/8R, 12R/12R, 10R/11R, 11R/13R and 7R+TCT/13R. The rare alleles 8R, 10R and 13R were not included in the comparison tests.

DISCUSSION

Because PCOS constitutes the most common cause of anovulatory infertility and hirsutism, several attempts have been made in order to determine the presence of causal mutations or recurrent polymorphisms in various genes that intervene on the synthesis of androgenic precursors (Gharani *et al.* 1997). The data have demonstrated that CYP11α gene pentanucleotide TTTTA repeat polymorphism is moderately associated with PCOS in Greece women (Diamanti-Kandarakis *et al.* 2000), but not in Hirsute women (Gaasenbeek *et al.* 2004). A case and control association study has indicated that CYP11α gene pentanucleotide TTTTA repeat polymorphism is weakly associated with BMI in the patients with PCOS in a population of Han Chinese women (Wang *et al.* 2006). Study in the population of young women with hyperandrogenism (97% of them were Caucasian in ethnic origin, the remainder being Indian or Afro-Caribbean) suggested that common variation at the aromatase gene (and not just rare loss-of-function mutations) is associated with androgen excess in girls and young women (Petry *et al.* 2005). Another study involved four candidates genes in Caucasian population with PCOS indicated that there was no association of CYP19 gene and PCOS (Tucci *et al.* 2001).

We have carried out the genetic association study of the CYP19 tetranucleotide TTTA repeat polymorphism in the forth intron in Han Chinese women with PCOS. In the present study, we have performed the genotyping experiments with advanced fluorescent capillary electrophoresis protocol. We found allele 11R was the most common one and the frequency of 11R in the patients with PCOS was lower than in the controls, but the *p*-value was the borderline statistically significance. We also found that the carriers with allele 11R among the patients with PCOS had decreased CHO compared to the patients carrying other alleles. the carriers with allele 7R-TCT among the patients with PCOS had increased CHO and LDL compared to the patients carrying other alleles. Both alleles may confer the genetic influence to the metabolic features in the development of PCOS in Han Chinese women.

Although we have limited knowledge whether gene expression is regulated by intronic polymorphisms, the similar evidence that an intronic SNP influences expression of the calpain 10 gene has also reported (Su 2001). Therefore, it may be necessary to further investigate the biological role of the specific alleles of this polymorphism in the CYP19 intron.

The results of the studies on the association of CYP19 gene and PCOS are different (Tucci *et al.* 2001; Jakubowski 2005; Söderlund *et al.* 2005). And we didn't found allele 9R in the population we studied, while the repeats of TTTA is from 7 to 13 in previous study of premenopausal women in Greece (Baghaei *et al.* 2003). Taking the previous and present study together, we suggest that this polymorphism is an ethnic and racial variant, which differently distributes the genotypes in the populations of European Caucasians and Han Chinese women.

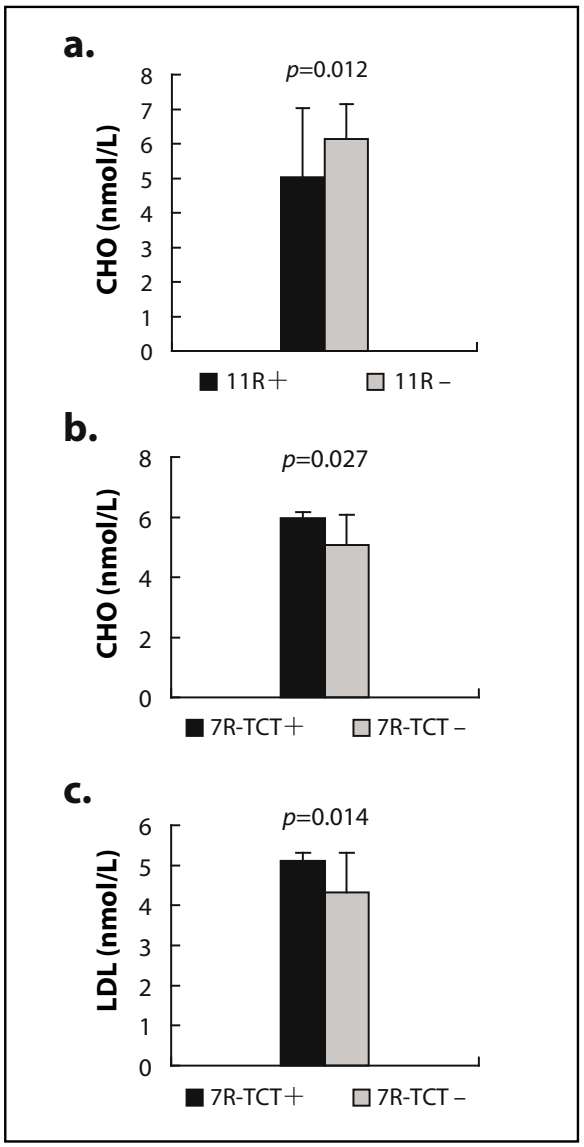

Fig. 1. Comparison analyses between the PCOS patients carrying with and without alleles 11R or 7R-TCT in the CYP19 tetranucleotide TTTA repeat polymorphism. The PCOS carriers with allele 11R had decreased CHO (p=0.012) compared to the patients with other alleles.The carriers with allelle 7R-TCT had elevated CHO (p=0.027) and LDL (p=0.014).

CONCLUSION

The most common allele of the tetranucleotide TTTA repeat polymorphism in the forth intron of CYP19 gene in the population of Han Chinese women is 11R, which was different with the previous study in European Caucasians. Allele 11R may be associated with PCOS in the population of Han Chinese women, and it may refrain from the hypercholesteremia of PCOS patients. Allele 7R-TCT may be related to the hypercholesteremia and

higher low-density lipoproteinemia of PCOS patients. This polymorphism is an ethnic and racial variant and moderately contributes to the pathogenesis of PCOS.

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REFERENCES

1 Baghaei F, Rosmond R, Landén M, Westberg L, Hellstrand M, Holm G, Eriksson E, *et al.* (2003). Phenotypic and genotypic characteristics of women in relation to personality traits. Int J Behav Med. **10**(4): 365–378.
2 Carlsson E, Poulsen P, Storgaard H, Almgren P, Ling C, Jensen CB, Madsbad S, *et al.* (2005). Genetic and nongenetic regulation of CAPN10 mRNA expression in skeletal muscle. Diabetes. **54**: 3015–3020.
3 Chen SA, Besman MJ, Sparkes RS, Zollman S, Klisak I, Mohandas T, Hall PF, *et al.* (1988). Human aromatase: cDNA cloning, Southern blot analysis, and assignment of the gene to chromosome 15. DNA. **7**(1): 27–38.
4 Christian RC, Dumesic DA, Behrenbeck T, Oberg AL, Sheedy PF 2nd & Fitzpatrick LA (2003). Prevalence and predictors of coronary artery calcification in women with polycystic ovary syndrome. J Clin Endocrinol Metab. **88**: 2562–2568.
5 Diamanti-Kandarakis E, Bartzis MI, Bergiele AT, Tsianateli TC, Kouli CR (2000). Microsatellite polymrophism (tttta) at –528 base pairs of gene CYP11a infleunecs hyperandrogenemia in patients with polycystic ovary syndrome. Fertile Steril. **73**: 735–741.
6 Diamanti-Kandarakis E, Kandarakis H, Legro RS (2006). The role of genes and enviroment in the etiology of PCOS. Endocrine. , **30**: 19–26.
7 Franks S, McCarthy MI, Hardy K (2006). Development of polycystic ovary syndrome: involvement of genetic and environmental factors. Int J Andrology. **29**: 278–285.
8 Fratantonio E, Vicari E, Pafumi C & Calogero AE (2005). Genetics of polycystic ovarian syndrome. Reprod Biomed Online. **10**: 713–720.
9 Gaasenbeek M, Powell BL, Sovio U, Haddad L, Gharani N, Bennett A, Groves CJ, *et al.* (2004). Large-scale analysis of the relationship between CYP11A promoter variation, polycystic ovarian syndrome, and serum testosterone. Clin Endocrinol Metab. **89**: 2408–2413.
10 Gharani N, Waterworth DM, Batty S, White D, Gilling-Smith C, Conway GS, McCarthy M, *et al.* (1997). Association of the steroid synthesis gene CYP11a with polycystic ovary syndrome and hyperandrogenism. Hum Mol Genet. **6**: 397–402.
11 Ito Y, Fisher CR, Conte FA, Grumbach MM and Simpson ER (1993). Molecular basis of aromatase deficiency in an adult female with sexual infantilism and polycystic ovaries. Proc Natl Acad Sci USA. **90**: 11673–11677.
12 Jakimiuk AJ, Weitsman SR, Brzechffa PR, Magoffin DA (1998). Aromatase mRNA expression in individual follicles from polycystic ovaries. Mol Hum Reprod. **4**(1): 1–8.
13 Jakubowski L (2005). Genetic aspects of polycystic ovary syndrome. Endokrynol Pol. **56**(3): 285–93.
14 Legro RS (2003). Polycystic ovary syndrome and cardiovascular disease: a premature association? Endocr Rev. **24**: 302–312.
15 Ovalle F & Azziz R (2002). Insulin resistance, polycystic ovary syndrome and type 2 diabetes mellitus. Fertil Steril. **77**: 1095–1105.
16 Petry CJ, Ong KK, Michelmore KF, Artigas S, Wingate DL, Balen AH, Zegher F, *et al.* (2005). Association of aromatase (CYP 19) gene variation with features of hyperandrogenism in two populations of young women. Hum Reprod. **20**(7): 1837–1843.
17 Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Hinshelwood MM, Graham-Lorence S, Amarneh B, *et al.* (1994). Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. Endocr. Rev. **5**(3): 342–355.
18 Su Y. Polycystic ovary syndrome: In Zhang L (2001). Clinical reproductuve endocrinology. Beijing, Chinese Scientific. pp: 367–399.
19 Söderlund D, Canto P, Carranza-Lira S, Méndez JP (2005). No evidence of mutations in the P450 aromatase gene in patients with polycystic ovary syndrome. Hum Reprod. **20**(4): 965–969.
20 Tucci S, Futterweit W, Concepcion ES, Greenberg DA, Villanueva R, Davies TF, Tomer Y (2001). Evidence for association of polycystic ovary syndrome in caucasian women with a marker at the insulin receptor gene locus. J Clin Endocrinol Metab. **86**(1): 446–449.
21 Wang Y, Wu X, Cao Y, Yi L, Chen J (2006). A microsatellite polymorphism (tttta)n in the promoter of the CYP11a gene in Chinese women with polycystic ovary syndrome. Fertil Steril. **86**: 223–226.