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Original Article

Association between polycystic ovary syndrome (PCOS) and *CYP11A1* polymorphism in Hainan, China: a case-control study

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Abstract: Polycystic ovary syndrome (PCOS) is one of the most common female endocrine disorders and a leading cause of female subfertility. The cytochrome P-450 11A1 (*CYP11A1*) gene encodes the key enzyme that catalyzes the initial and rate-limiting step in steroid hormone synthesis. In this study, the associations between seven single nucleotide polymorphisms (SNPs) in *CYP11A1* and PCOS susceptibility were examined. Seven SNPs in *CYP11A1* were genotyped using the MassARRAY iPLEX platform in 285 PCOS patients and 299 control individuals. Associations between the SNPs and the risk of PCOS were tested using various genetic models (co-dominant, dominant, recessive, and additive). Logistic regression models were used to derive odds ratios (ORs) and 95% confidence intervals. By χ^2 test, the differences of genotype frequencies of rs4887139 ($P = 0.035$) and rs4886595 ($P = 0.046$) were significant statistically difference between PCOS patients and controls under recessive model. In the genetic model analyses, we also found that the genotype "GG" of rs4887139 ($P = 0.035$) and genotype "CC" of rs4886595 ($P = 0.04$) were significant associated with increased the risk of PCOS by recessive model. We found four SNPs (rs12917295, rs11632698, rs1484215, and rs6495096) constructed four haplotypes ("CACG", "CATC", "CACC", and "GGCC") in the *CYP11A1* gene and none of the haplotype was significantly associated with risk of PCOS.

Keywords: Polycystic ovary syndrome (PCOS), cytochrome P-450 11A1 (*CYP11A1*), single nucleotide polymorphisms (SNPs), case control study

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous hormonal disorder affecting a fifteenth in women, and is one of the most common causes of female infertility. PCOS is a kind of reproductive and metabolic disorder characterized by hyper-androgen and insulin resistance, which affects 5.6% of reproductive aged women in Chinese and 6-8% in Caucasian [1, 2]. PCOS is a complex multifactor disorder and genetic factors have been implicated in its pathogenesis [3]. However, it's now widely accepted that genetic factors play an indispensable role in the development of PCOS and several candidate genes have been reported recently [4, 5].

Exposure to endogenous sex hormones has been reported as a risk factor for PCOS. The *CYP11A1* (cytochrome P-450 11A1) gene is located at 15q23-q24 and consists of nine exons spanning a total of 29,864 bp [6]. This gene encodes the cholesterol side chain cleavage enzyme P450scc, a member of the cytochrome P450 superfamily of enzymes, which resides on the mitochondrial inner membrane and catalyzes the conversion of cholesterol to pregnenolone, the initial and rate-limiting step in steroid hormone synthesis [7]. *CYP11A1* is primarily expressed in steroidogenic tissues, such as the adrenal cortex, gonads, and placenta. Although P450scc is always active, genetic variants of *CYP11A1* may alter its expression and activities, and thus result in certain hormone-related diseases. Polymorphisms of *CYP11A1* have been detected as potential markers in different hormone-dependent diseases, including breast cancer [8], prostate

Table 1. Characteristics of 285 POCS cases and 299 healthy individuals in this study

Characteristic	Case (N = 285)	Control (N = 299)	*p*-value
Mean BMI	20.48	20.61	0.585
< 24	267 (93.7)	274 (91.6)	
≥ 24	18 (6.30)	25 (8.4)	
Mean Age	28.5	32.66	< 0.01*
Mean age of menarche	14.50±1.86	14.4±1.3	0.619
The average menstrual cycle	29.0±3.96	29.0±2.4	0.832
Menstrual	4.93±1.36	5.00±1.53	0.54
Number of pregnancy	1.37±1.14	2.17±1.19	< 0.01*

*Indicates statistical significance $P < 0.05$. POCS: polycystic ovary syndrome.

cancer [9], endometrial cancer and PCOS [10, 11].

Previous association studies between *CYP11A1* and PCOS have focus on the (TTTTA)n microsatellite polymorphism in the promoter of *CYP11A1* [9, 12-15]. However, single nucleotide polymorphisms (SNPs) of *CYP11A1* have less been reported [16]. In this study, we chose seven SNPs in *CYP11A1* (rs12917295, rs11632698, rs1484215, rs6495096, rs4887139, rs9806234, and rs4886595) and investigate their association with PCOS.

Materials and methods

Study population

This Hainan region population-based case-control study comprising 285 PCOS patients diagnosed from January 2010 to May 2014 from the Hainan Province People's Hospital and 299 random samples of unrelated healthy individuals women on the same time from the health centers of Hainan Province People's Hospital were conducted.

PCOS diagnostic criteria

PCOS were diagnosed by the revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome [17, 18]. Simply, at least two of following three criteria was required: Clinical and/or biochemical sign of hyperandrogenism, oligomenorrhea or anovulation, and polycystic ovarian morphology on ultrasound. Meanwhile, other diseases which could cause hyperandrogenism should be excluded, such as hypothyroidism, congenital adrenal hyperplasia, Cushing's syndrome, androgen-secreting tumors, etc.

Demographic clinical data and ethic support

For each participant, a standardized epidemiological questionnaire including residential region, weight, height, age, ethnicity, education status, number of pregnancies, age of menarche, and family history of cancer was used to collect personal data through in-person interviews. Additionally, all the controls had normal menstrual cycles (less than 32 days) and normal body mass index (BMI), excluding hyperandrogenism, hirsutism, diabetes mellitus, etc. BMI was calculated as body weight in kilograms divided by square of height in meters. Venous blood samples (5 ml) and signed informed consent were obtained from each participant. All blood samples were quickly frozen in liquid nitrogen and stored at -80°C. This study was approved by the Hainan Province People's Hospital, and informed consent was obtained from all the women prior to inclusion.

SNP selection and genotyping

Using the HapMap database, seven candidate SNPs (rs12917295, rs11632698, rs1484215, rs6495096, rs4887139, rs9806234, rs4886595) in *CYP11A1* with minor allele frequencies > 5% in the Asian population and previously published associations with PCOS or steroidogenic enzymes were selected by previous studies [10, 16, 19]. The phenol-chloroform extraction method was performed to extract genomic DNA from whole blood [20]. The concentration of DNA was measured by spectrometry (DU530 UV/VIS™ spectrophotometer, Beckman Instruments, Fullerton, CA, USA). We used the Sequenom MassARRAY® Assay Design 3.0 Software to design a Multiplexed SNP MassEXTEND® assay [21]. SNP genotyping using the standard protocol recommended by the manufacturer was performed using Sequenom MassARRAY® RS1000. The SequenomTyper 4.0 Software™ was used to perform data management and analyses [22].

Statistical analysis

Differences in demographic characteristics between the POCS cases and controls were

Table 2. Basic information of the seven SNPs and Chi-square under the recessive model

SNPs	Gene	Position	Allele A/B	Case		Control		Pearson Chi-Square	*p*-Pearson Chi-Square
				AA count	AB-BB count	AA count	AB-BB count		
rs12917295	*CYP11A1*	74632284	G/C	4	281	6	293	0.315	0.574
rs11632698	*CYP11A1*	74637867	G/A	4	281	6	293	0.315	0.574
rs1484215	*CYP11A1*	74640109	T/C	29	256	24	275	0.816	0.366
rs6495096	*CYP11A1*	74640647	G/C	48	237	34	263	3.496	0.061
rs4887139	*CYP11A1*	74661945	G/A	37	248	23	276	4.429	0.035*
rs9806234	*CYP11A1*	74663033	A/G	18	267	21	278	0.117	0.732
rs4886595	*CYP11A1*	74663265	C/A	8	277	2	297	3.963	0.046*

A/B, minor/major alleles. *$P < 0.05$ indicates statistical significance.

evaluated by the Chi-square test (for categorical variables) or the Student's t-test (for continuous variables). Four unconditional logistic regression genetics models (dominant, co-dominant, recessive, and log-additive genetic models) were used to evaluate the association of each of the SNPs with POCS by SNP Stats [23] (Catalan Institute of Oncology, Barcelona, Spain), a web-based software http://bioinfo.iconcologia.net/snpstats/start.htm. We used Microsoft Excel (Microsoft Corp, Redmond, WA, United States) and the SPSS 18.0 Statistical Package (SPSS, Chicago, IL, USA) to perform statistical analyses. 95% confidence intervals (CIs) and Odds ratios (ORs) were calculated using unconditional logistic regression analyses adjusted for age and BMI. All *p* values reported in this study were two-tailed, and *p* values less than 0.05 were considered statistically significant. Linkage disequilibrium (LD) of the candidate SNPs was analyzed using HaploView v4.2 (Mark Daly's Laboratory, Massachusetts Institute of Technology/Harvard Broad Institute, Cambridge, MA, USA) [24]. Pairwise LD and haplotype constructions were performed using the SHEsis Software (http://analysis.bio-x.cn/myAnalysis.php) [25].

Results

Characteristics of the study participants

A total of 584 participants, including 285 PCOS cases and 299 controls, were successfully genotyped for further analysis. All of participants were women and at least 18 years old. There is significant ($P < 0.05$) difference between the cases and controls in terms of mean age distribution (28.5 in cases vs 32.6 in controls) and the number of pregnancy distribution (1.37 ± 1.14 in cases vs 2.17 ± 1.19 in controls). Additionally, BMI, mean age of menarche, the average menstrual cycle and menstrual were equally distributed among PCOS cases and control subjects (**Table 1**).

Association between the SNPs and PCOS risk

Table 2 summarizes the basic characteristics of the seven SNPs in the *CYP11A1*, and showed the minor allele and major allele of each SNP. We compared the differences in frequency distributions of alleles between cases and controls by Pearson χ^2 test under the recessive model and found only the minor allele "G" in rs4887139 ($P = 0.035$) and "C" of rs4886595 ($P = 0.046$) were significantly associated with risk.

We also used SNP Stats to assess the association between these SNPs and PCOS risks using four genetic models (co-dominant, dominant, recessive, and log-additive) by unconditional logistic-regression analysis. As shown in **Table 3**, we also found the genotype "G/G" of rs4887139 was associated with increased PCOS risk with OR = 1.79, 95% CI = 1.04-3.10, $P = 0.035$, and the genotype "CC" of rs4886595 was associated with increased PCOS risk with OR = 4.29, 95% CI = 0.90-20.36, $P = 0.04$ by the log-additive model.

One haplotype block that included four SNPs (rs12917295, rs11632698, rs1484215, and rs6495096) with D' = 1 (**Figure 1**) was detected in *CYP11A1* SNPs by haplotype analyses. Haplotypes with frequencies > 1% which including "CACG", "CATC", "CACC", and "GGCC" were

Table 3. Single SNPs association with PCOS (adjusted by BMI and age)

SNPs	Model	Genotype	Control	Case	OR (95% CI)	*P*-value	AIC	BIC
rs12917295	Co-dominant	C/C	210 (70.2%)	215 (75.4%)	1	0.36	813.2	826.3
		G/C	83 (27.8%)	66 (23.2%)	0.78 (0.53-1.13)			
		G/G	6 (2%)	4 (1.4%)	0.65 (0.18-2.34)			
	Dominant	C/C	210 (70.2%)	215 (75.4%)	1	0.16	811.3	820
		G/C-G/G	89 (29.8%)	70 (24.6%)	0.77 (0.53-1.11)			
	Recessive	C/C-G/C	293 (98%)	281 (98.6%)	1	0.57	812.9	821.7
		G/G	6 (2%)	4 (1.4%)	0.70 (0.19-2.49)			
	Log-additive	---	---	---	0.78 (0.56-1.09)	0.15	811.2	819.9
rs11632698	Co-dominant	A/A	210 (70.2%)	215 (75.4%)	1	0.36	813.2	826.3
		A/G	83 (27.8%)	66 (23.2%)	0.78 (0.53-1.13)			
		G/G	6 (2%)	4 (1.4%)	0.65 (0.18-2.34)			
	Dominant	A/A	210 (70.2%)	215 (75.4%)	1	0.16	811.3	820
		A/G-G/G	89 (29.8%)	70 (24.6%)	0.77 (0.53-1.11)			
	Recessive	A/A-A/G	293 (98%)	281 (98.6%)	1	0.57	812.9	821.7
		G/G	6 (2%)	4 (1.4%)	0.70 (0.19-2.49)			
	Log-additive	---	---	---	0.78 (0.56-1.09)	0.15	811.2	819.9
rs1484215	Co-dominant	C/C	149 (49.8%)	139 (48.8%)	1	0.66	814.4	827.6
		T/C	126 (42.1%)	117 (41%)	1.00 (0.71-1.40)			
		T/T	24 (8%)	29 (10.2%)	1.30 (0.72-2.33)			
	Dominant	C/C	149 (49.8%)	139 (48.8%)	1	0.8	813.2	821.9
		T/C-T/T	150 (50.2%)	146 (51.2%)	1.04 (0.75-1.44)			
	Recessive	C/C-T/C	275 (92%)	256 (89.8%)	1	0.37	812.4	821.2
		T/T	24 (8%)	29 (10.2%)	1.30 (0.74-2.29)			
	Log-additive	---	---	---	1.08 (0.84-1.39)	0.55	812.9	821.6
rs6495096	Co-dominant	C/C	119 (40.1%)	111 (39%)	1	0.16	808.9	822
		C/G	144 (48.5%)	126 (44.2%)	0.94 (0.66-1.33)			
		G/G	34 (11.4%)	48 (16.8%)	1.51 (0.91-2.52)			
	Dominant	C/C	119 (40.1%)	111 (39%)	1	0.78	810.5	819.2
		C/G-G/G	178 (59.9%)	174 (61%)	1.05 (0.75-1.46)			
	Recessive	C/C-C/G	263 (88.5%)	237 (83.2%)	1	0.061	807.1	815.8
		G/G	34 (11.4%)	48 (16.8%)	1.57 (0.98-2.51)			
	Log-additive	---	---	---	1.15 (0.91-1.46)	0.25	809.3	818
rs4887139	Co-dominant	A/A	240 (80.3%)	222 (77.9%)	1	0.071	810	823.1
		A/G	36 (12%)	26 (9.1%)	0.78 (0.46-1.34)			
		G/G	23 (7.7%)	37 (13%)	1.74 (1.00-3.02)			
	Dominant	A/A	240 (80.3%)	222 (77.9%)	1	0.48	812.8	821.5
		A/G-G/G	59 (19.7%)	63 (22.1%)	1.15 (0.77-1.72)			
	Recessive	A/A-A/G	276 (92.3%)	248 (87%)	1	0.035*	808.8	817.5
		G/G	23 (7.7%)	37 (13%)	1.79 (1.04-3.10)			
	Log-additive	---	---	---	1.20 (0.93-1.54)	0.15	811.2	820
rs9806234	Co-dominant	G/G	161 (53.9%)	158 (55.4%)	1	0.9	815.1	828.2
		G/A	117 (39.1%)	109 (38.2%)	0.95 (0.68-1.34)			
		A/A	21 (7%)	18 (6.3%)	0.87 (0.45-1.70)			
	Dominant	G/G	161 (53.9%)	158 (55.4%)	1	0.7	813.1	821.9
		G/A-A/A	138 (46.1%)	127 (44.6%)	0.94 (0.68-1.30)			
	Recessive	G/G-G/A	278 (93%)	267 (93.7%)	1	0.73	813.1	821.9

		A/A	21 (7%)	18 (6.3%)	0.89 (0.47-1.71)			
	Log-additive	---	---	---	0.94 (0.72-1.22)	0.65	813.1	821.8
rs4886595	Co-dominant	A/A	222 (74.2%)	203 (71.2%)	1	0.11	810.9	824
		C/A	75 (25.1%)	74 (26%)	1.08 (0.74-1.57)			
		C/C	2 (0.7%)	8 (2.8%)	4.37 (0.92-20.83)			
	Dominant	A/A	222 (74.2%)	203 (71.2%)	1	0.41	812.6	821.3
		C/A-C/C	77 (25.8%)	82 (28.8%)	1.16 (0.81-1.68)			
	Recessive	A/A-C/A	297 (99.3%)	277 (97.2%)	1	0.04*	809	817.8
		C/C	2 (0.7%)	8 (2.8%)	4.29 (0.90-20.36)			
	Log-additive	---	---	---	1.24 (0.89-1.73)	0.2	811.6	820.4

*$P < 0.05$ indicates statistical significance. Abbreviations: OR, odds ratio; CI confidence interval. SNP, single nucleotide polymorphism; AIC, Akaike's information criterion; BIC, Bayesian information criterion.

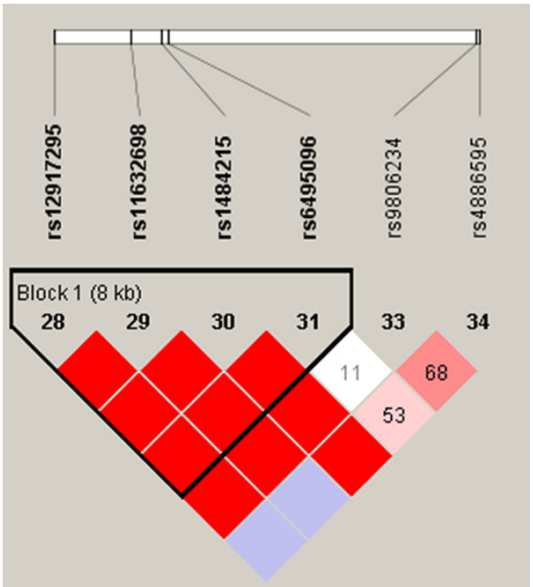

Figure 1. Linkage disequilibrium plots containing six SNPs from *CYP11A1*. Red squares display statistically significant associations between a pair of SNPs, as measured by D'; darker shades of red indicate higher D'.

selected for analyze, and the association between inferred haplotypes and PCOS risk among the individuals was analyzed. However, we found no association between the selected haplotype and PCOS risk (**Table 4**).

Discussion

In this study, we found the frequency of rs4887139 and rs4886595 were different between PCOS and healthy controls in Hainan region. Due to PCOS were not easy to get pregnant, the number of pregnancy among PCOS were significant less than healthy individuals. Further genetics model analysis found the minor of rs4887139 was protective allele, and the minor allele "C" of rs4886595 was a risk allele.

Ovarian steroidogenesis begins with the conversion of cholesterol into pregnenolone that is catalyzed by P450scc enzyme, an initial and rate-limiting step at the start of steroid hormone biosynthetic pathway. Several SNPs of *CYP11A1* have been reported to be involved in the aetiopathogenesis of PCOS that may alter testosterone levels. Numerous studies have examined *CYP11A1* gene polymorphisms, but most have focus on the pentanucleotide [TTTTA]n repeat (D15S520) located 528 bp upstream of the translational initiation site, which showed six common polymorphisms with four, six, seven, eight, nine, or ten repeats. In the Chinese population, the main variants are the four-, six-, and eight-repeat alleles, which are correlated with risks of breast cancer [26] and polycystic ovarian syndrome [10]. Although *CYP11A1* polymorphism was associated with breast cancer and POCS, mechanism was different. Estrogen is closely related and breast cancer [27], androgen is associated with PCOS. So far single nucleotide polymorphisms (SNPs) of *CYP11A1* have less been reported. However, PCOS cannot be associated with a single factor of this gene, more research on predisposing causes of PCOS and the function of *CYP11A1* gene is needed in the future.

Some limitations of the present replication study should be mentioned. First, the sample size of this replication study was relatively small and this replication study maybe not sufficient to detect the potential association between

Table 4. Haplotype association with response (adjusted by age and BMI)

Haplotype	Freq.	Case, Control Ratio Counts	Case, Control Freq	Chi-Square	*p*-value	OR (95% CI)	*p*-value
CACG	0.371	220.8:349.2, 212.8:385.2	0.387, 0.356	1.243	0.265	1.00	---
CATC	0.297	173.8:396.2, 172.8:425.2	0.305, 0.289	0.357	0.550	0.99 (0.74-1.33)	0.94
CACC	0.185	100.2:469.8, 116.2:481.8	0.176, 0.194	0.664	0.415	0.79 (0.57- .11)	0.18
GGCC	0.145	74.0:496.0, 95.0:503.0	0.130, 0.159	1.988	0.159	0.76 (0.52-1.11)	0.16

Abbreviations: Freq, Frequency; OR, odds ratio; CI confidence interval.

CYP11A1 gene and PCOS. Second, only seven SNPs were chosen which may cause incomplete coverage of the gene variations. Third, the recruited subjects were all Han Chinese women and the result could not represent other population.

Future studies should focus on early detection of the predisposing risk factors in PCOS development. Large and more genome-wide association studies devoted solely to PCOS among different populations will be necessary to identify new candidate genes and proteins that are involved in PCOS risk.

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Disclosure of conflict of interest

None.

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