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Original Article

THADA gene variants and polycystic ovary syndrome in a Hainan Chinese population

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Abstract: Background: Polycystic ovary syndrome (PCOS), the most common disorder in females, is characterized by a range of clinical complications and constitutes a potentially serious health threat worldwide. Previous genome-wide association studies have identified multiple susceptibility loci for PCOS; however, validation of these findings is still needed. Methods: We genotyped 10 single nucleotide polymorphisms in 285 Hainan Chinese PCOS patients and 299 control subjects and evaluated gene variants' association with risk of PCOS. Results: When adjusting for age and body mass index, we found two alleles in the *THADA* gene significantly associated with PCOS in our patient group: the "C" allele of rs13429458 as identified by dominant (OR, 1.44; 95% CI, 1.01-2.05; $P = 0.043$) and additive (OR, 1.35; 95% CI, 1.01-1.81; $P = 0.043$) genetic model. We also identified the "T" allele of rs12478601 as significant based on dominant model analysis (OR, 1.43; 95% CI, 1.01-2.01; $P = 0.041$). Conclusion: Our findings provide further evidence that certain genetic variations in *THADA* are associated with increased risk of PCOS among Hainan women.

Keywords: Single nucleotide polymorphism (SNP), polycystic ovary syndrome (PCOS), *THADA*

Introduction

Polycystic ovary syndrome (PCOS) is a hyperandrogenic, menstrual, and ovulatory disordered syndrome and is the most commonly documented endocrinopathy in women of reproductive age [1]. The condition affects 5-8% of the population and is characterized by oligomenorrhea, chronic anovulation, hyperandrogenism, enlarged cystic ovaries, infertility, obesity, and several cardiometabolic abnormalities, including metabolic syndrome, insulin resistance, Type 2 diabetes, dyslipidemia, atherosclerosis and hypertension [2, 3]. Despite remarkable efforts and recent research advancements, the complete pathomechanism of PCOS is not known, and no treatments for these women have been developed or tested in humans.

Although the basic etiology of PCOS has not been completely elucidated, several findings suggest that both genetic and environmental factors are involved in its pathogenesis [4]. Observational studies have identified specific environmental factors that contribute to the risk of PCOS, such as increased caloric intake, endocrine disruptors, and lifestyle [5, 6]. However, accumulating evidence indicates that PCOS is largely determined by hereditary factors and has strong familial clustering [7]. To date, candidate gene studies have identified many genes involved in molecular pathways and mechanisms possibly linked to PCOS, such as central energy metabolism, insulin secretion and action, gonadotropin action, and steroid hormone biosynthesis [8, 9]. In addition, previous genome-wide association studies (GWAS) have identified multiple susceptibility loci for PCOS in a Han Chinese population, including Chromosome 9q33.3 *DENND1A* loci, the 2p16.3 *LHCGR* locus, and the 2p21 *THADA* locus [10].

It is not known whether the same polymorphism may play different roles in PCOS susceptibility

Table 1. Demographic characteristics of study subjects by case-control status

	Cases	%	Controls	%	*P*[a]
Total	285		299		
Mean ± SD					
Age (years)	28.50 ± 6.858		32.66 ± 7.018		<0.001*
BMI (kg/m^2)	20.49 ± 2.713		20.612 ± 2.801		0.585
<24	267	93.7	274	91.6	
≥ 24	18	6.3	25	8.4	

BMI, body mass index. [a]Independent-samples. T-test. *Statistically significant values (*P*<0.05).

in different populations, as PCOS is a complicated multifactor genetic disease, and may be influenced by different genetic backgrounds. In our current study, we evaluated 10 single nucleotide polymorphisms (SNPs) previously identified by a Han Chinese GWAS with respect to PCOS development in case-control studies of similar population. Here, we tried to provide evidence for the genetic relationship between certain genetic variants and increased risk of PCOS.

Materials and methods

Study participants

We consecutively recruited 285 PCOS patients and 299 ethnically matched controls that were seen at Hainan Provincial People's Hospital in Hainan, China between January 2010 and October 2015. All subjects were associated with a single geographic location of Haikou and had to be stable residents in the area. Among patients, transvaginal ultrasound scan was performed to assess ovarian volumes and the number of follicles. All PCOS patients were recently diagnosed and confirmed to suffer from PCOS according to the 2003 Rotterdam Criteria, in which two of the following three conditions were present: clinical hyperandrogenism (hirsutism), anovulation, and polycystic ovary upon ultrasound examination. Participants had not received any systemic treatment before the time of examination and did not suffer any relevant diseases, such as Cushing's syndrome, thyroid dysfunction or hyperprolactinemia. Exclusion criteria for healthy subjects included congenital adrenal hyperplasias, 21-hydroxylase deficiency, central nervous system-related diseases, chronic diseases and conditions involving vital organs (liver, heart, lung and brain) and severe endocrine, metabolic, or nutritional diseases. The use of data and samples from study participants was approved by the Human Research Committee for Approval of Research Involving Human Subjects.

Clinical data and demographics

Upon recruitment, written informed consent was obtained from each participant informed consent, and data regarding demographic information, physiological characteristics, living habits, and biochemical tests were collected during interviewers. Body Mass Index (BMI) was calculated and overweight defined as BMI ≥ 24 Kg/m^2 based on the current Chinese recommended classification [11, 12]. Blood samples were taken from the study participants the morning after an overnight fast of at least 12 h.

SNP selection and genotyping

SNPs of the *THADA* gene with minor allele frequency (MAF) > 0.05 in the HapMap Asian population were obtained from previously published polymorphisms associated with PCOS [10, 13] and were applied to an initial screening. DNA was extracted from cells that had been cultured overnight using the phenol-chloroform extraction method described previously [14]. The DNA concentration was determined by spectrometry (DU530UV/VIS spectrophotometer, Beckman Instruments, Fullerton, CA, USA). A multiplexed SNP Mass EXTEND assay was designed with Sequenom Mass ARRAY Assay Design 3.0 Software, and SNP genotyping was performed utilizing the Sequenom Mass ARRAY RS1000 recommended by the manufacturer [15]. Sequenom Typer 4.0 Software was used to perform SNP data management and analyses [15, 16].

Statistical analysis

Statistical analysis was carried out in Microsoft Excel and SPSS 16.0 (SPSS, Chicago, IL, USA). All hypothesis testing were two-sided with a *p* value of 0.05 deemed as significant. The age data are presented as means and standard deviation (SD). Hardy-Weinberg equilibrium (HWE) was tested by a goodness-of-fit χ^2 test to compare observed and expected genotype fre-

Table 2. Detailed information of 10 SNPs examined in the *THADA* gene

SNP ID	Chr.	Gene	Position	Region	Alleles	HWE *P* Value	*P*[a]	OR (95% CI)
rs7567607	2	*THADA*	43638185	Intron	T/C	0.293	0.226	1.16 (0.91-1.48)
rs13029250	2	*THADA*	43638712	Intron	T/G	0.476	0.718	0.94 (0.67-1.32)
rs13429458	2	*THADA*	43638838	Intron	C/A	0.255	0.054	1.32 (1.00-1.75)
rs7582497	2	*THADA*	43638947	Intron	C/T	0.360	0.226	1.16 (0.91-1.48)
rs7605725	2	*THADA*	43641627	Intron	G/A	0.326	0.104	1.23 (0.96-1.57)
rs6746064	2	*THADA*	43717650	Intron	C/T	0.702	0.243	1.15 (0.91-1.46)
rs17334980	2	*THADA*	43718593	Intron	C/T	0.008	0.627	0.82 (0.37-1.82)
rs12478601	2	*THADA*	43721508	Intron	T/C	0.285	0.089	1.23 (0.97-1.57)
rs13022691	2	*THADA*	43723977	Intron	C/T	0.687	0.233	1.16 (0.91-1.48)
rs7563949	2	*THADA*	43725221	Intron	G/A	0.014	0.813	0.92 (0.44-1.89)

SNP, single-nucleotide polymorphism; Chr, Chromosome; OR, odds ratio; 95% CI, 95% confidence interval; HWE, Hardy-Weinberg equilibrium. [a]Two-sided χ^2 tests/Fisher's exact tests.

Table 3. Association of rs13429458 with PCOS risk based on logistic tests

	Case		Controls		*P*[a]	Logistic Regression Analysis		
	No.	%	No.	%		Adjusted OR	95% CI	*P*[b]
Genotype								
AA	168	58.9	200	66.9	0.138	1		0.120
CA	100	35.1	85	28.4		1.41	0.97-2.05	
CC	17	6.0	14	4.7		1.64	0.75-3.61	
Dominant								
AA	168	59.0	200	66.9	0.047*	1		
CA+CC	117	41.0	99	33.1		1.44	1.01-2.05	0.043*
Recessive								
AA+CA	268	94.0	285	95.3	0.490	1		
CC	17	6.0	14	4.7		1.46	0.67-3.17	0.340
Additive								
AA	168	58.9	200	66.9	0.060	1.35	1.01-1.81	0.043*
CA	100	35.1	85	28.4				
CC	17	6.0	14	4.7				

[a]Two-sided χ^2 tests/Fisher's exact tests. [b]Wald test adjusted for age and sex. *Statistically significant values ($P<0.05$).

quencies among cases and controls. Odds ratios (ORs) and 95% confidence intervals (CI) were estimated using unconditional logistic regression models with adjustment for age and body mass index (BMI) [17, 18]. The genotypic associations were analyzed with the dominant, recessive, and additive genetic models. Finally, we used the Haploview software package (http://www.broadinstitute.org/haploview) and the SHEsis software platform (http://www.nhgg.org/analysis/) to analyze the linkage disequilibrium (LD) structure, calculate D' to define haplotype blocks and estimate haplotype frequencies [19, 20].

Results

Demographic characteristics and candidate SNPs

Two hundred ninety-nine healthy controls (aged 28.50 ± 6.858 years) and 285 patients with PCOS (aged 32.66 ± 7.018 years) participated in the study. **Table 1** shows the demographic characteristics of all participants. Detailed information of the 10 SNPs examined within the *THADA* gene is listed in **Table 2**. Each SNP was required to have a call rate greater than or equal to 95%, and each SNP was tested for departures from Hardy-Weinberg equilibrium. We found that variants rs17334980 and rs7563949 significantly deviated from HWE and therefore were excluded from the analysis. We compared differences in frequency distributions of alleles between cases and controls with a χ^2 test and did not find any SNPs nominally significant at the 5% level.

Significant SNPs

Genotype distributions and allele frequencies of SNPs rs13429458 and rs12478601 in PCOS cases and healthy controls are shown in **Tables 3** and **4**. To test the association of *THADA* gene polymorphisms with PCOS, genotypic ORs were calculated. After adjustment for

Table 4. Association of rs12478601 with PCOS risk based on logistic tests

	Case		Controls		P^a	Logistic Regression Analysis		
	No.	%	No.	%		Adjusted OR	95% CI	P^b
Genotype								
CC	115	40.3	144	48.2	0.165	1		0.120
TC	133	46.7	121	40.5		1.41	0.98-2.03	
TT	37	13.0	34	11.3		1.5	0.86-2.62	
Dominant								
CC	115	40.4	144	48.2	0.058	1		
TC+TT	170	59.6	155	51.8		1.43	1.01-2.01	0.041*
Recessive								
CC+TC	248	87.0	265	88.6	0.051	1		
TT	37	13.0	34	11.4		1.27	0.75-2.14	0.370
Additive								
CC	115	40.3	144	48.2	0.094	1.28	1.00-1.65	0.054
TC	133	46.7	121	40.5				
TT	37	13	34	11.3				

[a]Two-sided χ^2 tests/Fisher's exact tests. [b]Wald test adjusted for age and sex. *Statistically significant values (P<0.05).

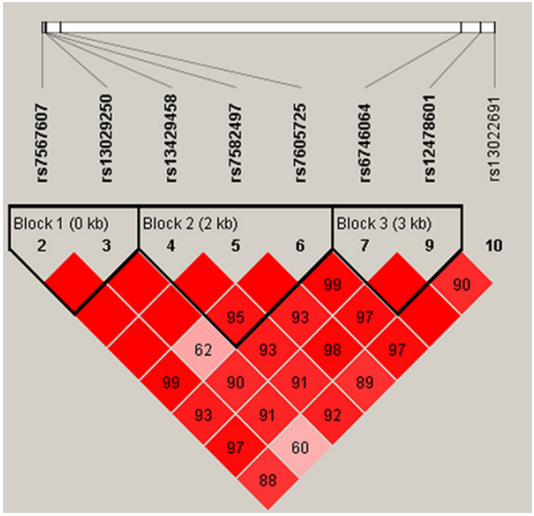

Figure 1. Haplotype block map for all the SNPs of the *THADA* gene.

age and BMI, our results showed that rs13429458 was significantly associated with susceptibility to PCOS according to the dominant (OR, 1.44, 95% CI, 1.01-2.05, P = 0.043) and additive (OR, 1.35, 95% CI, 1.01-1.81, P = 0.043) models. A second significant SNP (rs12478601) was identified by dominant model analysis (OR, 1.43; 95% CI, 1.01-2.01; P = 0.041).

Linkage disequilibrium

The candidate SNPs in the *THADA* gene showing strong linkage are found in **Figure 1**. To date, we have not found any significant association between *THADA* haplotypes and risk of PCOS (**Table 5**).

Discussion

In the current study, we explored 10 previously reported PCOS-associated loci identified through GWAS in a Chinese Han population. Two susceptibility loci from chromosome 2 (rs13429458 and rs12478601) were significantly associated with increased risk of PCOS. To our knowledge, this study is to report validation of these loci in a Hainan population.

THADA, mapped to chromosomal band 2p21, encodes thyroid adenoma-associated protein, which is expressed in the pancreas, thyroid, testes, thymus, adrenal medulla, adrenal cortex, small intestine, and stomach. In addition, its expression may be involved in death receptor pathway and apoptosis [21, 22]. Moreover, *THADA* is the target gene affected by cytogenetic rearrangement in benign thyroid adenomas [23]. Because *THADA* is of fairly large size (370 kb) and is controlled by many regulatory elements, variants within *THADA* have been associated with a range of diseases [24]. Two SNPs (rs13429458 and rs12478601) are located in introns of *THADA* and were previously reported to be associated with PCOS [10]. Chen et al. [10] found that rs13429458 was significantly associated with PCOS at genome-wide significance and in a large sample size, which was confirmed and extended in experiments performed by Shi et al. [13], Zhao et al. [25] and Cui et al. [26, 27]. Our data support these findings reported previously of an association between rs13429458 and increased PCOS risk in Chinese populations. Interestingly, other studies identified no correlation between rs13429458 and PCOS in Caucasian women [21, 28-31]. We also found an association between rs12478601 and PCOS susceptibility,

Table 5. Haplotype frequencies of SNPs in the *THADA* genes and the association with PCOS risk in case and control subjects

Gene	Haplotype	Freq (case)	Freq (control)	p^a	Crude		Adjusted[b]	
					OR (95% CI)	*p*	OR (95% CI)	*p*
THADA	Block: rs7567607-rs13029250							
	CG	0.638	0.673	0.209	1		1	
	TG	0.230	0.188	0.076	1.28 (0.96-1.69)	0.093	1.33 (0.99-1.79)	0.062
	TT	0.132	0.139	0.719	1.01 (0.72-1.41)	0.980	1.02 (0.71-1.45)	0.930
	Block: rs13429458-rs7582497-rs7605725							
	ATA	0.632	0.671	0.167	1		1	
	CCG	0.225	0.184	0.082	1.27 (0.95-1.70)	0.100	1.33 (0.98-1.81)	0.065
	ACG	0.106	0.107	0.956	1.06 (0.72-1.54)	0.780	1.09 (0.73-1.61)	0.680
	ACA	0.026	0.033	0.455	0.84 (0.43-1.64)	0.600	0.82 (0.40-1.66)	0.580
	Block: rs6746064-rs12478601							
	TC	0.619	0.652	0.243	1		1	
	CT	0.363	0.316	0.089	1.20 (0.94-1.53)	0.140	1.26 (0.97-1.62)	0.079
	CC	0.018	0.032	0.118	0.58 (0.26-1.27)	0.180	0.66 (0.29 - 1.50)	0.320

[a]Two-sides χ^2 test/Fisher's exact tests. [b]Adjusted for age and sex in a logistic regression model.

which supports the findings of Chen et al. [10], but these results are contrary to studies of this variant in Caucasian PCOS patients [21, 31]. These varying results may be explained by several factors. First, various genetic backgrounds and environments exist among ethnicities and individuals, and the same SNP loci have different genotype frequencies and may interact with other genetic variants in unexpected ways according to ethnicity. Secondly, studies have shown a clear pattern of disparity in clinical manifestations of PCOS patients' component phenotypes across ethnic populations [32]. In addition, uncontrolled environmental factors and gene-environment factors interactions involved in PCOS phenotype development may contribute to the inconsistent findings. Moreover, recruitment of cohorts and diagnostic criteria used, as well as varying sample sizes, also contributed to the heterogeneity. Thus, determinations of PCOS genetic susceptibility gene variants might be associated with different populations.

Potential limitations of our study should be mentioned. We did not use Bonferroni correction for multiple comparisons because the method might have increased the chance of a type II error. Subgroup analyses, such as stratification by age, geographical area, and risk profile could not be performed because of the small sample size in this study. Furthermore, all of our study participants were aboriginal inhabitants of Haikou, so, replication of the current study in a larger cohort of genetically heterogeneous patients should be pursued.

In summary, we identified a significant association between increased PCOS risk and both the rs13429458 "C" allele and the rs12478601 "T" allele of the *THADA* gene in a Hainan population. Future functional studies are needed to confirm the correlation between the *THADA* gene and PCOS pathogenesis, especially with respect to different ethnicities. We predict that identification and characterization of additional PCOS susceptibility genes will ultimately provide more efficient strategies for the diagnosis, prevention, and treatment of PCOS in genetically heterogeneous populations.

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Disclosure of conflict of interest

None.

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