Family-Based Association Study of rs17300539 and rs12495941 Polymorphism in Adiponectin Gene and Polycystic Ovary Syndrome in a Chinese Population

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Data Collection B
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Background:
Polycystic ovary syndrome (PCOS) is a complex disease that has both genetic and environmental components. Adiponectin plays an important role in the regulation of insulin sensitivity and insulin resistance (IR) in PCOS. The aim of this study was to determine 2 single-nucleotide polymorphisms (SNPs) variants (rs12495941 and rs17300539) of the adiponectin gene (ADIPOQ) in polycystic ovary syndrome (PCOS) families.

Material/Methods:
We recruited 197 PCOS probands, their biological parents, and 192 controls. Anthropometric variables, including hip circumference (HC) and waist circumference (WC), were measured in all subjects during their first visit to the outpatient department. Serum T, FBG, FINS, TC, TG, LDL, and HDL levels were measured. PCOS patients were divided into 2 groups based on BMI: group A (BMI <25 kg/m^2) and group B (BMI ≥25 kg/m^2). Parents of PCOS were accordingly categorized into group C and group D (fathers), and group E and group F (mothers). The associations among ADIPOQ rs12495941, rs17300539, and PCOS were analyzed using the transmission disequilibrium test (TDT).

Results:
A significant association was found between SNP rs17300539 and PCOS in our Chinese population. The levels of TG and FINS and the genotype frequencies of rs17300539 are significantly different between overweight and lean PCOS. No significant association was detected for rs12495941.

Conclusions:
TDT confirms that rs17300539 of ADIPOQ is strongly associated with the risk of PCOS in a Chinese Han population, but rs12495941 of ADIPOQ is not associated with the occurrence of PCOS.

MeSH Keywords:
Adiponectin • Polycystic Ovary Syndrome • Polymorphism, Single Nucleotide

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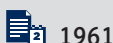
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Background

Affecting 5-10% or more of women at reproductive age, polycystic ovary syndrome (PCOS) is the most common endocrine and metabolic disorder [1,2]. There has been a research on the metabolic disorders in PCOS patients in recent studies, which leads to cardiovascular events, dyslipidemia, and insulin resistance [3–6]. Numerous clinical studies subsequently found the link of insulin resistance with PCOS, characterizing the significant insulin resistance in PCOS patients [7–10]. Insulin resistance is considered as the leading cause of PCOS widely [11].

It is showed in recent study that adiponectin plays a key role in modulation insulin sensitivity [12]. The adiponectin gene (ADIPOQ) is located at chromosome 3q27. In this chromosome region, genome-wide scans reveal a susceptibility locus for type 2 diabetes mellitus (T2DM), obesity and coronary heart disease. A number of recent studies have investigated the associations between multiple polymorphisms of ADIPOQ and PCOS risk. Two single-nucleotide polymorphisms (SNPs) was focused in most of these studies: rs2241766 and rs1501299 [13–18]. Up to now, there is no related report about the association between PCOS and rs17300539, rs12495941, which both also relates to T2DM. In order to overcome the problem of population stratification, we performed a family-based genetic analysis to assess the association between the 2 SNPs rs17300539, rs12495941 of ADIPOQ and PCOS.

Material and Methods

Ethics

This study was approved by the Ethics Committee of Taishan Medical University and Shandong University (Ethics approval number 200719). All participants signed the informed consent.

Materials

Participants with PCOS were recruited from the Center for Reproductive Medicine, Hospital Affiliated to Taishan Medical University and Provincial Hospital Affiliated to Shandong University from June 2007 to April 2013. According to the 2003 Rotterdam criteria, PCOS was diagnosed [19]. We enrolled 197 unrelated PCOS patients and their biological parents (591 participants in total) in the study. PCOS patients were divided into 2 groups depending on body mass index (BMI) as follows: A (BMI <25 kg/m^2) and B (BMI ≥25 kg/m^2). Their age ranged from 18 to 38 years, mean (SD) age, 27.26 (3.41) years. Fathers of PCOS patients were categorized into groups C and D with respective to patients' BMI. Mothers of patients were likewise categorized into groups E and F.

The control group included 192 women recruited during the same period. Their age ranged from 23 to 44 years, and the mean (SD) age was 32.11 (4.5) years. All of them were healthy, without polycystic ovaries on vaginal ultrasound, with regular menstrual cycles, and without evidence of acne, alopecia, hirsutism, or endocrine dysfunction, and had not received hormonal therapy (including oral contraceptives) during the last 3 months.

Clinical and biochemical measurements

Anthropometric variables, including weight, body height, hip circumference (HC), and waist circumference (WC), were measured for all participants during their first visit to the hospital. BMI was calculated as the weight (kg) divided by the square of body height (m^2), and waist-hip ratio (WHR) as WC/HC.

Fasting venous blood samples were collected between 8: 00 AM and 10: 00 AM after a 12-h overnight fast. Fasting glucose (FG) was measured by the oxidase method using an AU640 automatic biochemistry analyzer (Olympus Company, Hamburg, Germany). Testosterone (T) and fasting insulin (FINS) were detected by chemiluminescence immunization. Serum triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL), and low-density lipoprotein (LDL) were detected using an Ft-7060 precipitation and enzymatic analyzer (Beckman Coulter Inc., Galway, Ireland).

Genotyping

Blood samples for molecular genetic studies from all 783 participants were collected in tubes containing ethylene diamine tetraacetic acid (EDTA) as an anticoagulant and were stored at −20°C. According to the protocol of the QIAamp DNA mini kit (QIAGEN, Hilden, Germany), genomic DNA was extracted from whole blood. Polymerase chain reaction (PCR) was performed with appropriate primers after genomic DNA was obtained, with the forward primer 5'-TAGTGAGCCGAGATTGTGC-3' and the reverse primer 5'-TCCTTAGGCA TGTAGCTTTCC-3' for rs12495941, forward primer 5'-ACTCTGCTGAGA TGGACGGA -3' and reverse primer 5'-GGGATGAGGGTGAAGATGGG-3' for rs17300539. The PCR conditions were as follows: denaturation at 95°C for 5 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 45 s; and a final extension at 72°C for 7 min. At the end of the cycles, the products of PCR were analyzed by melting curve, and then sequenced using an ABI PRISM 310 automated sequencer (Applied Biosystems, Foster City, CA). All the samples were double-genotyped with 100% concordance.

Statistical analyses

Descriptive statistics for SNP were obtained from Haploview 4.2, which includes Hardy-Weinberg equilibrium and minor allele frequency (MAF) [20]. Then, association between PCOS and

Table 1. Results of the TDT obtained with two SNPs of 197 PCOS family trios.

Marker ID	Overtransmitted allele	T	Not-T	Total TDT	Transmission frequency	TDT χ^2	P-value
Rs17300539	G	98	71	169	0.580	4.314	0.0378
Rs12495941	G	106	94	200	0.530	0.72	0.3961

T – number of transmissions in TDT analysis.

Table 2. Comparison of parameters between obese and lean PCOS.

	PCOS patients of BMI <25 kg/m²	PCOS patients of BMI ≥25 kg/m²	P
Age (years)	26.56±3.22	28.32±3.43	<0.001
H (cm)	161.75±5.01	161.77±4.81	0.987
W (cm)	57.52±6.15	75.63±9.63	<0.001
WC (cm)	79.35±8.26	94.02±9.68	<0.001
HC (cm)	93.28±7.08	103.69±6.55	<0.001
WHR	0.85±0.06	0.91±0.06	<0.001
T (ng/dl)	58.28±23.98	66.06±26.80	0.051
FG (mmol/l)	5.68±1.21	5.63±1.61	0.803
TC (mmol/l)	4.61±1.11	4.66±0.94	0.715
HDL (mmol/l)	1.52±0.55	1.35±0.47	0.068
LDL (mmol/l)	2.65±0.95	2.80±0.96	0.296
TG (mmol/l)	1.08±0.80	1.37±0.69	0.01
FINS (mIU/L)	10.42±7.36	15.29±7.80	0.001

WC – waist circumference; HC – hip circumference; WHR – waist hip ratio; T – testosterone; FG – fasting glucose; TC – total cholesterol; HDL – high-density lipoprotein cholesterol; LDL – low-density lipoprotein; TG – triglycerides; FINS – fasting insulin.

the SNP was analyzed by transmission disequilibrium test (TDT) performed on Haploview 4.2. Statistical analysis was performed using SPSS 15.0 (SPSS, Chicago, IL, USA). Statistical significance was considered at the two-tailed P level of 0.05. Descriptive data are reported as mean ± standard deviation (SD). Categorical data are expressed as percentages and frequencies. The differences in clinical and biochemical variables among groups were evaluated by the independent samples t-test. Genotypic and allelic distributions were compared using the Pearson's chi-squared test. Logistic regression analysis and Bonferroni modification were used to adjust for confounding factors such as age, HC, and biochemical variables. P<0.05 was considered statistical significance.

Results

TDT in PCOS family

A total of 197 families were involved in the analysis. The SNPs were highly polymorphic in our samples with MAF of 0.406 (rs12495941) and 0.273 (rs17300539). The genotype distributions of the 2 SNPs were in Hardy-Weinberg equilibrium (p>0.05). Significant differences in transmission were found for rs17300539 (transmitted/non-transmitted 98: 71; χ^2=4.314; P=0.0378). Regarding the other SNP, rs12495941, no significant difference was found between transmission and non-transmission (106: 94; χ^2=0.72; P=0.3961). LD was analyzed in the study: rs17300539 and rs12495941 were not in LD (r^2=0.0010; D'=0.064).

Clinical and metabolic characteristics

After categorizing PCOS patients by BMI, the clinical and metabolic characteristics of the PCOS patients and their parents are shown in Tables 1 and 2. Age, WC, HC, WHR, TG, and FINS were all significantly different between group A and group B (P<0.05) (Table 2), while between groups C and D, only age, WC, and TC were significantly different (P<0.05, Table 3). Between groups E and F, significant differences were found in age, weigh, BMI, WC, HC, and FINS (P<0.05, Table 3). The genotype frequencies of rs12495941 and rs17300539 were not significantly different

Table 3. Comparison of parameters of obese and lean PCOS' parents.

	Fathers of PCOS patients with BMI <25 kg/m²	Fathers of PCOS patients with BMI ≥25 kg/m²	P	Mothers of PCOS patients with BMI <25 kg/m²	Mothers of PCOS patients with BMI ≥25 kg/m²	P
Age (years)	53.39±5.28	55.18±6.00	0.029	52.11±4.84	53.64±5.01	0.034
H (cm)	170.35±5.58	170.81±5.20	0.562	159.37±4.98	159.06±4.59	0.669
W (cm)	71.53±11.03	74.42±11.24	0.077	63.83±9.79	68.47±11.25	0.003
BMI	24.62±3.40	25.48±3.50	0.092	25.05±3.21	26.65±4.98	0.007
WC (cm)	87.33±10.67	90.46±9.82	0.046	86.23±9.16	90.00±11.04	0.011
HC (cm)	96.44±8.16	98.71±7.69	0.061	97.61±7.48	100.97±8.17	0.004
WHR	0.91±0.07	0.92±0.05	0.297	0.88±0.060	0.89±0.06	0.457
FG (mmol/l)	6.49±2.25	6.70±2.42	0.523	6.06±1.35	6.11±1.31	0.779
TC (mmol/l)	4.93±0.96	5.28±1.14	0.02	5.36±1.00	5.36±0.92	0.972
HDL (mmol/l)	1.44±0.58	1.43±0.49	0.912	1.58±0.59	1.65±0.50	0.512
LDL (mmol/l)	2.97±0.87	3.16±1.10	0.183	3.18±0.90	3.10±0.83	0.531
TG (mmol/l)	1.45±1.13	1.55±0.99	0.540	1.38±1.01	1.48±1.00	0.517
FINS (mIU/L)	6.91±3.79	7.77±5.77	0.464	8.99±4.10	12.03±7.48	0.034

Table 4. Comparison of genotype frequencies between obese and lean PCOS and their parents.

		A (%)	B (%)	P	C (%)	D (%)	P	E (%)	F (%)	P
Rs17300539	AA	67 (55.8)	46 (59.7)		57 (47.5)	43 (55.8)		62 (51.7)	37 (48.1)	
	GA	49 (40.8)	25 (32.5)	0.242	60 (50)	29 (37.7)	0.131	53 (44.2)	32 (41.6)	0.229
	GG	4 (3.3)	6 (7.8)		3 (2.5)	5 (6.5)		5 (4.2)	8 (10.4)	
Rs12495941	GG	49 (40.8)	22 (28.6)		39 (32.5)	23 (29.9)		47 (39.2)	26 (33.8)	
	GT	55 (45.8)	43 (55.8)	0.216	60 (50.0)	36 (46.8)	0.600	59 (49.2)	44 (57.1)	0.54
	TT	16 (13.3)	12 (15.6)		21 (17.5)	18 (23.4)		14 (11.7)	7 (9.1)	

between overweight and lean PCOS patients. Similar results were observed between parents of overweight and lean PCOS patients (p>0.05, Table 4). Similarly, no significant difference in genotype or allele frequencies was observed between PCOS patients and the control group (p>0.05, Table 5). After adjustment for age, HC, and other biochemical factors, we observed that WC, FINS, and TG remained significantly different between groups A and B (Table 6). In groups C and D, with adjustment for age and other variables, the results became less significant (P>0.05, Table 7). Similar results were found in groups E and F (Table 7). After Bonferroni modification, we found FINS in group F was significantly higher compared with the other 3 groups (P<0.05), and BMI and HC of group F were significantly greater compared with groups C and E (P<0.05).

Discussion

PCOS is a complex multifactorial disorder that may result from the interaction between protective and predisposing genomic variants under the effects of environmental factors. Several genetic association studies of ADIPOQ and PCOS have been conducted in recent years. Most studies focused on 2 SNPs – rs2241766 and rs1501299 – but rarely on other polymorphisms.

Rs12495941 is located in intron 1 of ADIPOQ and it is not involved in any putative transcription binding site. There has been relatively little study of rs12495941, and most existing studies focused on diabetes and cardiovascular diseases. Recent studies suggested that rs12495941 is significantly associated with the risk of T2DM [21], total body weight, waist

Table 5. Distribution of the rs12495941 in women with PCOS and controls.

Variables	PCOS (n=197)		Controls (n=192)		P
Age (years)	27.26±3.41		32.11±4.50		0.000
BMI (kg/m²)	24.73±4.23		24.05±3.77		0.093
Oligo- or amenorrhea	n=197 (100%)		n=0 (0.00%)		
Polycystic ovaries	n=195 (98.98%)		n=0 (0.00%)		
Hyperandrogenism	n=85 (43.15%)		n=0 (0.00%)		
Rs17300539 Genotype, n (%)					
AA	113	(57.4)	94	(49.0)	
GA	74	(37.6)	75	(39.1)	0.033
GG	10	(5.1)	23	(12)	
Alleles, n (%)					
A	300	(76.14)	263	(68.49)	
G	94	(23.86)	121	(31.51)	
Rs12495941 Genotype, n (%)					
GG	71	(36.00)	74	(38.50)	
GT	98	(49.70)	86	(44.8)	0.592
TT	28	(14.20)	32	(16.70)	
Alleles, n (%)					
G	240	(60.91)	234	(60.94)	
T	154	(39.09)	150	(39.06)	

Table 6. The coefficient of variation (%) of obese and lean PCOS.

	PCOS patients of BMI <25 kg/m²	PCOS patients of BMI ≥25 kg/m²
Age (years)	12.12	12.11
H (cm)	3.10	2.97
W (cm)	10.69	12.73
WC (cm)	10.41	10.30
HC (cm)	7.59	6.32
WHR	7.06	6.59
T (ng/dl)	41.15	40.57
FG (mmol/l)	21.30	28.60
TC (mmol/l)	24.08	20.17
HDL (mmol/l)	36.18	34.81
LDL (mmol/l)	35.85	34.29
TG (mmol/l)	74.07	50.36
FINS (mIU/L)	70.63	51.01

WC – waist circumference; HC – hip circumference; WHR – waist hip ratio; T – testosterone; FG – fasting glucose; TC – total cholesterol; HDL – high-density lipoprotein cholesterol; LDL – low-density lipoprotein; TG – triglycerides; FINS – fasting insulin.

Table 7. The coefficient of variation (%) of obese and lean PCOS' parents.

	Fathers of PCOS patients with BMI <25 kg/m²	Fathers of PCOS patients with BMI ≥25 kg/m²	Mothers of PCOS patients with BMI <25 kg/m²	Mothers of PCOS patients with BMI ≥25 kg/m²
Age (years)	9.89	10.87	9.29	9.34
H (cm)	3.28	3.04	3.12	2.89
W (cm)	15.42	15.10	15.33	16.43
BMI	13.81	13.74	12.81	18.69
WC (cm)	12.22	10.86	10.62	12.27
HC (cm)	8.46	7.79	7.66	8.09
WHR	7.69	5.43	6.82	6.74
FG (mmol/l)	34.67	36.12	22.28	21.44
TC (mmol/l)	19.47	21.59	18.66	17.16
HDL (mmol/l)	40.28	34.27	37.34	30.30
LDL (mmol/l)	29.29	34.81	28.30	26.77
TG (mmol/l)	77.93	63.87	73.19	67.57
FINS (mIU/L)	54.85	74.26	45.61	62.18

and hip circumference [22], stroke [23], higher plasma adiponectin level, and lower LDL-cholesterol levels, but not with adrenomedullin level [24]. Ong et al. [25] found that 4 genetic variants of ADIPOQ were associated with adiponectin level, and the effect was stronger for rs182052 and rs12495941. On the contrary, no association between rs12495941 and adiponectin level was found [26]. Rs17300539 is the other SNP of ADIPOQ. Recently, Lu et al. [27] suggested that with risk of obesity in people of white ethnicity, ADIPOQ rs17300539 are associated. In an African American cohort, An et al. [28] found that rs17300539 was associated with fasting glucose and plasma adiponectin levels. Gao et al. [29] found that in the expected direction, rs17300539 was strongly associated with serum adiponectin levels and insulin sensitivity. Similarly, after adjustment for plasma adiponectin, a nominally significant association was found with plasma insulin and HOMA-IR and ADIPOQ variant rs17300539 [30]. On the contrary, Han et al. [31] found no statistically significant associations between type 2 diabetes risk and -11391G>A (rs17300539), but there is no related report about the association between these 2 SNPs and PCOS. In the present family-based study, we conducted TDT to explore the possible association between rs12495941 and rs17300539 and PCOS. Rs17300539 showed a significant transmission difference, suggesting that rs17300539 is a risk marker for PCOS, whereas rs12495941 was not statistically associated with PCOS. To avoid the influence of age, BMI, and T, we performed a case-control study. We also found no difference in rs12495941 between PCOS patients and the control group, whereas rs17300539 showed

significant differences. Furthermore, rs17300539 GA and AA genotypes had an increased risk for PCOS compared with GG genotype. After adjustment for age, T, and BMI, the difference remained significant. Therefore, rs17300539 AA genotype appears to be a marker for increased risk of PCOS susceptibility. These results provide useful information for further functional studies. Large-sample studies are needed to investigate the association between ADIPOQ and PCOS.

The role of obesity in the pathophysiology of PCOS remains unclear. In this study, we found overweight PCOS women were older and had higher levels of WC, HC, WHR, TG, and FINS compared with lean PCOS women. The parents of overweight PCOS women had higher WC levels. In addition, fathers of overweight PCOS women had higher TC levels, while the mothers had higher weight, BMI, HC, and FINS levels (P<0.05). Thus, we speculate that the WC levels of parents, TC level of fathers, and weight, BMI, HC, and FINS level of mothers probably are associated with obesity of PCOS patients. After adjustment for age, HC, and other biochemical variables, we found WC and TG remained significantly different between group A and B (P<0.05), but no significant differences in any of the variables were found between group C and group D. Similar results were observed in groups E and F. After Bonferroni modification, we found that FINS in group F was significantly higher than in the other 3 groups (P<0.05), and that BMI and HC in group F were significantly greater compared with groups C and E (P<0.05). Likewise, no significant difference in the genotype frequencies

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of rs12495941 and rs17300539 existed in either obese or lean PCOS patients and their parents. Therefore, we think the BMI, HC, and FINS of mothers are more closely related with obesity in PCOS patients, and WC and TG levels of PCOS patients probably are directly associated with obesity.

Conclusions

Our study is the first investigation into the association of rs12495941 and rs17300539 with the risk of PCOS. TDT confirmed that rs17300539 in ADIPOQ is significantly associated with the risk of PCOS in a Chinese Han population. A limitation of our study is the small size, which perhaps resulted in

inconsistent findings. Studies with larger samples are needed to further investigate the association between other SNPs of ADIPOQ and PCOS.

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Conflict of interest

The authors have no conflicts of interest.

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