Family-Based Analysis of -675 4G/5G Polymorphism in the PAI-1 Gene in Chinese Polycystic Ovary Syndrome

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Research Article

Keywords: Polycystic ovary syndrome, 4G/5G polymorphism, transmission disequilibrium test, PAI-1 gene, single nucleotide polymorphism

Posted Date: June 21 st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-382013/v1>

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Abstract

Background: Insulin resistance (IR) is one of the main pathophysiology of PCOS. Previous studies have shown that 4G/5G polymorphism in promoter region of plasminogen activator inhibitor-1 gene (PAI-1) can affect insulin sensitivity by elevating the level and activity of plasma PAI-1, involved in the formation of IR. In order to elucidate the relationship between 4G/5G polymorphism of PAI-1 gene and PCOS, we used transmission disequilibrium test (TDT) to study the nuclear family of PCOS.

Purpose: This study used family-based analysis of TDT to determine whether an association exists between 4G/5G polymorphism of PAI-1 gene and PCOS and in Han Chinese in order to identify PAI-1 gene as a genetic susceptibility factor for PCOS.

Methods: Eight hundred and fifty-five participants consisting of 285 trios (mother, father and offspring with PCOS) were recruited from the Center for Reproductive Medicine, Shandong University, from July 2007 to August 2014. 4G/5G polymorphism of PAI-1 gene was genotyped using direct sequencing protocol and TDT was used to analyze the association between PAI-1 gene and PCOS.

Results: Though the 5G allele in PAI-1 gene was overtransmitted in families, no statistical significance existed and there was no association between PAI-1 gene and PCOS. The levels of FINS, HOMA-IR and TG were significantly different between obese and lean PCOS and the parents.

Conclusion: No significant evidence of association or linkage was found between 4G/5G polymorphism of PAI-1 gene and PCOS, indicating that PAI-1 gene was unlikely to play a major role in the etiology of PCOS in our sample.

Introduction

Polycystic ovary syndrome (PCOS) is a complex disease involving multiple organs, reproductive-endocrine and metabolic abnormalities, which affect up to 4%-18% of women of reproductive age (1, 2). The widely accepted pathophysiologic mechanisms were insulin resistance and hyperandrogenism, which could lead to a series of metabolic disorders and long-term complications such as type 2 diabetes mellitus, dyslipidemia, metabolic syndrome, premature arteriosclerosis and endometrial cancer, and so on (3–7). Epidemiology studies suggested that the complex interactions of genetic factors and environmental resulted in the arises of PCOS (8–10). A large number studies have shown that 4G/5G polymorphism in promoter region of Plasminogen activator inhibitor-1 gene (PAI-1) can affect insulin sensitivity by elevating the level and activity of plasma PAI-1, participating in the formation of insulin resistance (IR). Current studies make no consensus on the relationship between PAI-1 gene 4G/5G polymorphism and PCOS. In order to make further confirmation of relationship between PAI-1 gene 4G/5G polymorphism and PCOS, we use transmission disequilibrium test (TDT) to study the 285 nuclear family (mother, father and offspring with PCOS, 855 participants in total) of PCOS. TDT can guarantee the coherency of the all objects in same genetic background, eliminates the differences of traditional case-control study on genetic and environmental factors, and can eliminate the false positive results.

Materials And Methods

Subjects

A total of 855 participants, consisting of 285 trios (mother, father and offspring with PCOS) were recruited from the center for Reproductive Medicine, Shandong Provincial Hospital, Affiliated to Shandong University, between July 2007 to August 2014. They were all Han Chinese in Shandong province and signed Informed consent for molecular studies. The study was approved by the Ethics Committee of Shandong University. PCOS was diagnosed according to the 2003 Rotterdam criteria, at least two of the following three features: oligomenorrhea or amenorrhea, clinical or biochemical hyperandrogenism, and polycystic ovarian morphology on ultrasound. Other related disease, such as Cushing' syndrome, hypothyroidism or significant elevations of serum prolactin(PRL), non-classic 21-hydroxylase deficiency, adrenal congenital hyperplasia and estrogen-secreting tumors were excluded (11, 12). Based on body mass index(BMI), PCOS patients were divided into two groups: group A ($\text{BMI} \geq 25\text{kg/m}^2$) and group B ($< 25\text{kg/m}^2$). According to corresponding categories of PCOS patients, their parents were divided into group C versus D (fathers) and group E versus F (mothers) .

Methods

Anthropometrical variables, such as waist circumference(WC), hip circumference(HC), body height and weight were collected in all patients during the first visit to the outpatient department. Blood samples of patients with PCOS were obtained on days 3–5 of their menstrual cycle. All probands underwent a 75g oral glucose tolerance test. Fasting blood glucose (FBG) and fasting insulin (FINS) and 2-h insulin levels were detected using chemiluminescence immunoassay (endocrine hormone determination kit; Haier Import and Export, Ningbo, China) and 2-h glucose level were detected with the use of the oxidase method (AU640 automatic biochemistry analyzer; Olympus company, Hamburg, Germany), and the serum levels of follicle-stimulating hormone(FSH), luteinizing hormone(LH), total testosterone(TT) and estrogen were measured enzymatically on an automated biochemistry analyzer (Olympus 600, Clinical Chemistry Analyzer; Olympus Diagnostica GmbH, Co.Clare, Ireland). The homeostasis-model assessment for insulin resistance (HOMA-IR) was calculated according to the following equation: $\text{fasting blood glucose (FBG, mmol/L)} \times \text{fasting insulin (FINS, mU/L)} / 22.5$. Serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol concentrations were measured by the precipitation and enzymatic method (Ft-7060; Beckman Coulter, Galway, Ireland).

Genotyping

Whole-blood samples were collected in tubes for all participants containing ethylene diamine tetraacetic acid via peripheral venous puncture and stored at -20°C . Genomic DNA was extracted using a QIAamp DNA minikit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. The single nucleotide of -675 4G/5G in the PAI-1 gene was amplified by polymerase chain reaction (PCR) with pair of primers as following: for -675 4G/5G (Pubmed, locus rs1799889): 5'-GTCTGGACACGTGGGGG-3' (forward for 5G), 5'-GTCTGGACACGTGGGGA-3' (forward for 4G), 5'-TGC AGCCAGCCACGTGATTGTCTAG-3' (reverse for both

4G and 5G). The positional information of the – 675 4G/5G in the PAI-1 gene was shown in Table 2. Reactions were carried out under the following conditions: initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 30s, annealing for 30s at 60°C, extension at 72°C for 45s, and finally 72°C for 5 min. The polymerase chain reaction products were analyzed by melting curve first. Outliers lacking high peaks during melting curve were alternately analyzed by 1% agarose gel electrophoresis (AGE) and then sequenced on an automated sequencer (ABI PRISM 310; Applied Biosystems, Foster City, CA). All samples were double genotyped with 100% concordance.

Statistical analyses

Descriptive statistics for – 675 4G/5G polymorphisms of the PAI-1 gene, including minor allele frequency (MAF) and Hardy–Weinberg equilibrium, were obtained by Haploview 4.2 (12). Then the association between the – 675 4G/5G polymorphisms in the PAI-1 gene and PCOS was tested by the TDT analysis, which was carried out by Haploview 4.2 too. Statistical significance was considered at the two-tailed level of $P = 0.05$. TDT is a valid chi-squared test for the linkage hypothesis regardless of population history. In the TDT test, by collecting related PCOS family trios, we analyzed the difference between the probability of parents-to-offspring transmission and the hypothesis of no association (probability of transmission 50%). If the significant discrepancy existed, indicated an association between PAI-1 gene polymorphisms and PCOS.

The data of clinical and biochemical variables among different subgroups was analyzed by independent-samples t -test using the Statistical Package for Social Sciences (SPSS) version 22.0. Categorical data were expressed as frequencies and percentages. Descriptive characteristics were reported as mean $\pm$ SD. Genotypic and allelic distributions were compared using the Pearson chi-squared test and one-way analysis of variance in the PCOS group. $P < 0.05$ were considered statistically significant.

Results

Clinical and metabolic characteristics

As shown in Table 1, the average age of 285 PCOS patient was 27.14 years (27.29 ± 3.78) and the mean BMI was 25.33kg/m^2 (25.3 ± 4.60). The mean FBG and the 2-h glucose were 5.59 mmol/L (5.59 ± 1.11) and 6.85mmol/L (6.85 ± 2.73), respectively. The mean FINS and the 2-h INS were respectively 14.49mIU/L (14.49 ± 8.90) and 75.37mIU/L (75.37 ± 67.25). The HOMA-IR was 2.81 (2.81 ± 2.68).

The categorizing conditions of PCOS patients by BMI were shown in Table 2. We found significant differences in WC, HC, SBP, DBP, FINS, 2-h INS ($P < 0.0001$), F-G score, LDL cholesterol, HDL cholesterol, TG, LH, HOMA and SHBG ($P < 0.05$) between group A (PCOS patients of $\text{BMI} \geq 25\text{Kg/m}^2$) and group B (PCOS patients of $\text{BMI} < 25\text{Kg/m}^2$).

The clinical and metabolic characteristics of PCOS's parents were shown in Table 3. WC, HC, SBP, DBP, FINS, LDL, TG, HOMA were significant different Both in group C and D, E and F ($P < 0.05$).

Table 1. Clinical and metabolic characteristic of 285 PCOS Patients

	Mean	SD
Age(years)	27.29	3.78
BMI(kg/m ²)	25.33	4.60
WC(cm)	86.79	12.49
HC(cm)	98.71	10.69
SBP(mmHg)	120.42	15.48
DBP(mmHg)	79.62	11.85
F-G score	2.18	2.18
FBG(mmol/L)	5.59	1.11
2-h glucose(mmol/L)	6.85	2.73
FINS(mIU/L)	14.49	8.90
2-h insulin(mIU/L)	75.37	67.25
TC(mmol/L)	4.74	0.96
LDL cholesterol(mmol/L)	3.07	0.97
HDL cholesterol(mmol/L)	1.32	0.41
TG(mmol/L)	1.44	1.08
FSH(IU/L)	5.93	1.57
LH (IU/L)	11.20	6.18
PRL (ng/mL)	21.12	24.35
TSH (IU/L)	2.91	1.67
Oestroen (pg/mL)	52.95	40.36
TT(ng/mL)	56.82	30.48
DHEAS(mg/dL)	259.05	97.71
HOMA	2.81	2.68
INS-B (pg/mL)	57.01	28.96
AMH (ng/mL)	6.87	4.39
SHBG (nmol/L)	43.66	30.63

BMI=Body mass index; WC=Waist circumference; HC=Hip circumference; SBP=Systolic blood pressure; DBP=Diastolic blood pressure; F-G score=Ferriman-Gallwey score ; FBG=Fasting blood glucose; FINS=Fasting insulin; TC=Total cholesterol; LDL=low-density lipoprotein; HDL=high-densitylipoprotein; TG=Triglycerides; FSH=Follicle-stimulating hormone; LH=luteinizing hormone; PRL=prolactin;TSH=Thyroid-stimulating hormone; DHEAS=Dehydroepiandrosteronesulphate; TT=Total testosterone; HOMA-IR=homeostasis-model assessment insulin resistance index; INS-B=inhibin B ; AMH=anti-mullerian hormone; SHBG=sex hormone binding globulin.

Table 2. Comparison of Clinical and metabolic features of between obese and nonobese PCOS

Characteristic	Group A (n=123)	Group B (n=162)	P-value
Age(years)	27.51±4.52	26.93±3.64	0.2380
WC(cm)	96.34 ±9.57	78.29 ±7.76	<0.0001

HC(cm)	106.2 ±9.58	92.11 ±6.37	<0.0001
SBP(mmHg)	125.8 ±16.79	115.5 ±12.33	<0.0001
DBP(mmHg)	84.2 ±11.44	75.4 ±10.69	<0.0001
F-G score	1.93 ±1.88	2.38 ±2.42	0.002
FBG(mmol/L)	5.63 ±1.29	5.53 ±0.97	0.4740
2-h glucose(mmol/L)	7.18 ±2.78	6.48 ±2.69	0.0678
FINS(mIU/L)	18.91 ±9.80	10.24±5.11	<0.0001
2-h insulin(mIU/L)	101.2±73.03	51.59 ±51.03	<0.0001
TC(mmol/L)	4.81 ±0.90	4.66±1.02	0.2359
LDL cholesterol(mmol/L)	3.23 ±1.01	2.94±0.91	0.0193
HDL cholesterol(mmol/L)	1.22 ±0.29	1.39±0.49	0.0005
TG(mmol/L)	1.86 ±1.31	1.03±0.55	<0.0001
FSH(IU/L)	5.80 ±1.55	6.08 ±1.59	0.1934
LH (IU/L)	9.86±4.93	12.47±6.98	0.0016
PRL(ng/mL)	19.69 ±22.68	22.56±26.15	0.3984
TSH(IU/L)	3.11±1.94	2.72 ±1.35	0.0945
Oestroen (pg/mL)	49.32 ±41.66	55.98±39.91	0.2452
Total testosterone(ng/mL)	58.99 ±26.89	54.798±33.57	0.2754
DHEAS(mg/dl)	260.9 ±108.0	257.8 ±87.76	0.8268
HOMA	4.88 ±2.77	2.54±1.42	<0.0001
SHBG(nmol/L)	28.240±17.36	72.22±46.08	<0.0001

Note: Group A: PCOS patients of body mass index(BMI)≥25Kg/m²; Group B: PCOS patients of body mass index(BMI)<25Kg/m². BMI= Body mass index; WC=Waist circumference; HC=Hip circumference; SBP=Systolic blood pressure; DBP=Diastolic blood pressure; F-G score= Ferriman-Gallwey score ; FBG=Fasting blood glucose; FINS=Fasting insulin; TC=Total cholesterol; LDL=low-density lipoprotein; HDL=high-densitylipoprotein; TG=Triglycerides; FSH=Follicle-stimulating hormone ;LH=luteinizing hormone; PRL=prolactin; TSH= Thyroid-stimulating hormone; TT=Total testosterone; DHEAS= Dehydroepiandrosterone-sulphate; HOMA-IR=homeostasis-model assessment insulin resistance index; INS-B=inhibin B ; AMH=anti-mullerian hormone;SHBG=sex hormone binding globulin.

Table 3 Comparison of Clinical and metabolic features of between obese and lean PCOS'parents

Characteristic	Group C	Group D	P-value	Group E	Group F	P-value
Age(years)	52.89 ± 5.18	54.99±6.38	0.0032	52.67 ± 5.04	53.63±5.71	0.1375
WC (cm)	96.76 ±8.45	84.60 ±8.59	0.0001	92.71±8.68	81.12±7.65	0.0001
HC(cm)	104.0±6.78	95.24±7.87	0.0001	104.0±7.85	93.34±9.14	0.0001
SBP(mmHg)	142.5±19.6	132.6±19.85	0.0001	138.3±21.62	131.2±20.05	0.0053
DBP(mmHg)	92.28±14.3	83.89±12.04	0.0001	88.79 ±14.58	81.78±12.35	0.0001
FBG(mmol/L)	6.55 ±2.30	6.00±1.54	0.0703	6.05±1.37	5.95±1.38	0.6440
FINS(mIU/L)	9.99 ±6.46	5.05 ±2.20	0.0001	11.79±5.76	7.10±3.50	0.0001
TC(mmol/L)	5.26 ±1.00	5.09±1.10	0.3188	5.43±0.98	5.35±1.00	0.587
TG(mmol/L)	1.83 ±1.24	1.38±1.07	0.0145	1.76±1.30	1.28±0.93	0.007

HDL(mmol/L)	1.30 ±0.36	1.41±0.50	0.1670	1.42±0.46	1.55±0.51	0.1329
LDL (mmol/L)	3.38 ±1.15	3.06±0.90	0.055	3.30±0.88	3.22±1.00	0.5736
HOMA	3.20±2.49	1.30±0.61	0.0001	2.94±1.66	1.86±0.96	0.0004

Group C : fathers of women with PCOS with BMI≥25Kg/m²; Group D: fathers of women with PCOS with BMI < 25Kg/m² Group E: mothers of women with PCOS with BMI≥25Kg/m² Group F: mothers of women with PCOS with BMI < 25Kg/m².

BMI=Body mass index; WC=Waist circumference; HC=Hip circumference; SBP=Systolic blood pressure; DBP=Diastolic blood pressure; FBG=Fasting blood glucose; FINS=Fasting insulin; TC= Total cholesterol; LDL=low-density lipoprotein; HDL=high-density lipoprotein; TG= Triglycerides; HOMA-IR=homeostasis-model assessment insulin resistance index.

Clinical and metabolic characteristics of the patients with PCOS according to the genotype of -675 4G/5G polymorphisms(rs1799889) of the PAI-1 gene

The clinical and metabolic characteristics of the patients with PCOS according to the genotype of PAI-1 (rs1799889) were shown in Table 4, no significant differences were observed between the different genotypes.

Table 4 The characteristics of the patients with PCOS according to rs1799889

Variables	4G/4G	4G/5G	5G/5G	<i>F- values</i> ¹	4G/4G+4G/5G	<i>T-values</i> ²
Age(yeas)	27.13±3.26	27.25±4.02	27.65±3.97	0.3	27.20±3.74	-0.74
BMI	24.83±4.19	25.60±4.87	25.4±4.51	0.77	25.31±4.63	-0.12
WC (cm)	86.32±11.89	86.66±12.75	88.18±13.02	0.36	86.53±12.4	-0.83
HC(cm)	99.37±12.22	98.28±10.05	99.08±9.64	0.3	98.7±10.92	-0.23
SBP(mmHg)	118.71±13.64	121.27±15.37	123±17.07	1.4	120.3±14.75	-1.13
DBP(mmHg)	80.02±11.24	79.31±11.99	80.75±12.19	0.29	79.58±11.69	-0.62
FBG(mmol/L)	5.59±0.81	5.52±0.86	5.55±0.75	0.21	5.55±0.84	-0.07
2hGlu(mmol/L)	7.06±2.44	6.57±2.48	6.87±2.93	0.73	6.74±2.47	-0.29
FINS(mmol/L)	13.09±7.51	14.66±9.54	15.4±8.8	0.94	14.12±8.9	-0.84
2hins(mmol/L)	67.9±63.4	76.55±67.35	73.26±70.68	0.31	73.42±65.89	0.01
CHO(mmol/L)	4.86±1.03	4.64±0.92	4.74±0.95	1.3	4.72±0.97	-0.11
LDL(mmol/L)	2.99±1.05	3.02±0.91	3.18±1.07	0.58	3.01±0.96	-1.06
HDL(mmol/L)	1.41±0.49	1.31±0.42	1.31±0.44	1.15	1.35±0.45	0.54
TG(mmol/L)	1.32±0.84	1.41±1.06	1.46±0.98	0.34	1.38±0.99	-0.05
FSH(IU/L)	6.05±1.43	5.73±1.7	6.25±1.35	2.01	5.84±1.61	-1.53
LH(IU/L)	11.86±6.51	10.84±5.93	11.21±6.44	0.66	11.22±6.15	0.01
PRL(ng/mL)	21.38±23.84	22.45±28.38	17.26±7.77	0.71	22.07±26.8	2.01
TSH(IU/L)	3.2±3.91	2.85±1.55	4.07±6.37	1.68	2.97±2.61	-1.1
E2(pg/mL)	48.46±26.92	58.32±50.69	45.92±17.31	1.88	54.92±44.11	1.23
DHEAS	252.74±98.03	259.98±101.68	269.86±84.77	0.36	257.4±100.2	-0.72
HOMA	3.58±2.28	3.69±2.65	3.92±2.38	0.22	3.65±2.52	-0.61

¹ for comparison with 4G/4G, 4G/5G and 5G/5G group. ² for comparison with 4G/4G+4G/5G and 5G/5G group.

BMI=Body mass index; WC=Waist circumference; HC=Hip circumference; SBP=Systolic blood pressure; DBP=Diastolic blood pressure; F-G score=Ferriman-Gallwey score; FBG=Fasting blood glucose; FINS=Fasting insulin; TC=Total cholesterol; LDL=low-density lipoprotein; HDL=high-density lipoprotein; TG=Triglycerides; FSH=Follicle-stimulating hormone; LH=luteinizing hormone; PRL=prolactin; TSH=Thyroid-stimulating hormone; TT=Total testosterone; DHEAS=Dehydroepiandrosterone-sulphate; HOMA-IR=homeostasis-model assessment

insulin resistance index; INS-B=inhibin B; AMH=anti-mullerian hormone; SHBG=sex hormone binding globulin; Glu=glucose.

There were 123 obese PCOS patients and 162 lean PCOS patients classified by BMI. The distributions of genotypes of PAI-1(rs1799889)were shown inTable 5. In obese PCOS Group, the frequencies of genotypes 4G/4G, 4G/5G and 5G/5G were respectively 35.78%(44/123), 50.4%(62/123) and 13.82%(17/123). The corresponding proportions in lean PCOS were 27.78%(45/162), 53.09%(86/162) and 19.13%(31/162). The frequencies of allele 4G and 5G were 60.98% (150/246) and 39.02%(96/246) in obese PCOS patients, while 55.35% (176/318) and 44.65%(142/318) in lean PCOS patients. There was no significance difference in genotypes of PAI-1 between the two groups (chi-squared = 2.7002, P = 0.2592), and no significance difference in allele frequencies existed too (chi-squared = 1.8023, P = 0.018).

Table 5 Genotypes and allele frequencies of rs1799889 between obese and lean PCOS patients

	obese (n=123)	lean (n=162)	χ^2	P
Genotypes, n (%)				
4G/4G	44(35.78)	45(27.78)	2.7002	0.2592
4G/5G	62 50.4	86 53.09		
5G/5G	17 13.82	31 19.13		
Genotypes, n (%)				
4G	150 60.98	176 55.35	1.8023	0.1794
5G	96 39.02	142 44.65		

Hardy-Weinberg equilibrium Test

In our study, the gene transmission of PAI-1(4G/5G, rs1799889) coincided with Hardy-Weinberg equilibrium, as shown in Table 6 .

Table 6 The location of 4G/5G (PAI-1) and Hardy-Weinberg equilibrium

SNP	Position	HWE ^a	Alleles	MAF ^b
rs1799889	38802554	0.4742	4G:5G	0.325

a, HWE=Hardy-Weinberg equilibrium; b, MAF=minor allele

MAFs and TDT analysis

A total of 285 families were analyzed in our study. The excessive transmission existed through the - 675 4G/5G polymorphism analysis of PAI-1 gene. The transmission frequency was 0.523. The MAFs of -675 4G/5G polymorphisms (rs1799889) of the PAI-1 gene was 0.325 (P > 0.05). The TDT results for - 675 4G/5G polymorphisms(rs1799889) of the PAI-1 gene shown no association with PCOS (transmitted/non-transmitted = 134:124; χ^2 = 0.388; P = 0.5336).

Table 7 Positional information and results of the TDT obtained with 4G/5G SNP of 285 PCOS family trios

Variant	Position ^a Overtransmitted allele	T	Not- T	Total TDT	Transmission frequency	HWEp	MAF	TDT χ^2	P- value
rs1799889	38802554 5G	134	124	258	0.523	0.4742	0.325	0.388	0.5336

HWEp=Hardy-Weinberg equilibrium; MAF=minor allele frequency. T, number of transmissions in TDT analysis.

Discussion

As a complex and polygene related neuroendocrine disease, polycystic ovary syndrome (PCOS) is influenced by environmental and genetic factors, and show the general metabolic abnormalities and the abnormal ovarian local regulation. PCOS has short-term and long-term complications such as obesity, diabetes, dyslipidemia, infertility, and the risk of cardiovascular disease. Besides these, elevated activation of the blood coagulation system of PCOS has been reported (14).

PAI-1, was an important regulator of the endogenous fibrinolytic system. Recent studies reported that the 4G/5G polymorphism in the promoter of PAI-1 gene might relate to the increased risk of PCOS, while the conclusions were not consistent. Sales reported that PAI-1 4G/5G polymorphism might increase the susceptibility of PCOS in Brazil, Asians, Europe and Turkey, but not in Caucasian population (9, 15–16). Wang L reported that PAI-1 4G/5G polymorphism may be a major key in the pathogenesis of PCOS among chinses women (17). While TT Zhang had a contrary conclusion, their meta-analysis included a total of 11 case-control studies and found there were no association between PAI-1 4G/5G and PCOS risk under all models (18).

The discrepancy of the previous studies might ascribe to the population stratification and environmental factors. To avoid these possible impact factors, we conducted the 285 family trios and used the transmission disequilibrium test (TDT) to verify the linkage between PAI-1 4G/5G polymorphism and PCOS. Our data showed that the HWEp was 0.4742 and the MAFs of the PAI-1 4G/5G was 0.325 by the TDT with no statistical significance ($\chi^2 = 0.388$, $P = 0.5336$). We failed to find the association between PAI-1 4G/5G and PCOS. The conclusion was consistent with the results of TT Zhang (18). While our previous case-control studies showed that PAI-1 4G/5G polymorphism was associated with PCOS women in Han Chinese. The difference reminded us that we needed joint multicenter and collected more samples for further validation.

Michalska M reported that adipose tissue especially visceral adipose tissue could generate PAI-1 and led to an elevated blood level of PAI-1, speculating the association between obesity and PAI-1(19). Ma found that PAI-1 and obesity rats had a direct causal relationship (20). One study found that obese PCOS patients had higher blood PAI-1 levels and IR was more common than non-obese PCOS patients (9). Although the relation between obesity and incidence of PCOS has not been well elucidated, the close relationship between infertility, hyperandrogenism, glucolipid metabolic abnormalities and obesity are validated. Our study also concentrated on the relationship between PAI-1 and obesity, we divided the subjects into obesity and non-obesity, we found that WC, HC, SBP, DBP, FINS, LDL, TG, HOMA in obesity patients and their parents were higher than non-obesity, and FINS, 2-h insulin, HOMA significantly

increased especially ($P < 0.001$). while FBG had no signification between the two groups. The results verified that IR widely existed in both obese PCOS patients and their parents, and the relative insufficiency of insulin led to the compensatory increased insulin secretion and hyperinsulinemia. Additionally, we also speculated that obese of PCOS had family genetic predisposition.

Declarations

Funding

National Basic Research Program of China (2011CB944502)

Key Research and Development Project of Hainan Province (CN) (2012BAI32B04)

Key Program for Basic Research of the Science and Technology Commission of Shanghai Municipality, China (12JC1405800)

National Natural Science Foundation of China (81471428)

Conflicts of interest/Competing interests

There is no conflict of interest.

Availability of data and material

Data transparency

Code availability

Not applicable

Authors' contributions

Xiaocui Song was responsible for the design of total study and results analysis. Li Ge mainly concentrated on the collection of samples and the manuscript writing. Dongsha Wang, Li Li and Dongmei Ma were responsible for the data input and relevant information collection of PCOS trios.

Ethics approval

The study was approved by the Ethics Committee of Shandong University.

Consent to participate

All participants signed informed consent.

Consent for publication

All authors approved the publication of the manuscript.

Acknowledgements

We thank all PCOS patients and their families, and the staff of the center for Reproductive Medicine, Shandong Provincial Hospital, Affiliated to Shandong University.

Conclusion

In the 285 PCOS family trios, we studied the relationship between the PAI-1 4G/5G polymorphism and the pathogenesis of PCOS, however, we failed to find that 4G/5G significantly over-transmitted to PCOS proband from their parents. No enough evidences supported PAI-1 4G/5G gene polymorphism as a risk factor for the susceptibility of PCOS. Further family study in multicenter and multi-ethnic are necessary to reveal the association.

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