Association of single-nucleotide polymorphisms rs2197076 and rs2241883 of *FABP1* gene with polycystic ovary syndrome

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Received: 31 July 2015 / Accepted: 22 November 2015 / Published online: 9 December 2015
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Abstract

Purpose The objective of this study was to evaluate the association between single-nucleotide polymorphisms (SNPs) rs2197076 and rs2241883 in fatty acid-binding protein 1 (*FABP1*) gene and polycystic ovary syndrome (PCOS).

Methods The two alleles rs2197076 and rs2241883 in *FABP1* gene in 221 PCOS women and 198 normal women were amplified and sequenced. Allele frequency comparison was performed between the PCOS and control groups, and genotype-phenotype correlation analysis was performed using dominant and recessive models to assess the association of *FABP1* and the main features of PCOS.

Results Allele frequency analyses showed a strong association of SNPs rs2197076 and rs2241883 of *FABP1* gene with PCOS ($P < 0.001$). The additive, dominant, and recessive genotype model analyses further supported this association even

after adjusting for age and body mass index (BMI). The minor allele frequency (MAF) of rs2241883 in obese PCOS women was less than that in obese control women. Further genotype-phenotype correlation analysis showed that SNP rs2197076 had a stronger association with the main features of PCOS than SNP rs2241883.

Conclusion In the association of SNPs in *FABP1* gene with PCOS, rs2197076 was more closely associated with its main features than rs2241883 and seemed to play a more important role in the pathogenesis of PCOS.

Keywords Polycystic ovary syndrome · PCOS · Liver fatty acid-binding protein · *FABP1* gene · SNP · Lipid metabolism

Capsule Analysis of *FABP1* gene identified rs2197076 and rs2241883 polymorphisms involved in the pathogenesis of polycystic ovary syndrome in Chinese women.

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Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine-metabolic disorder, affecting 6–8 % women of childbearing age [1]. The condition is characterized by chronic oligoovulation and/or anovulation, hyperandrogenism, and polycystic ovarian morphology on ultrasonography [2]. Although women with PCOS have a high risk of suffering from metabolic syndrome [3], cardiovascular disease (CVD) [4], type 2 diabetes [5], insulin resistance [6], and obesity [7], its etiology and underlying pathophysiology still remain elusive. Several family and twin studies have revealed a genetic basis of the etiopathogenesis of PCOS [8, 9]. Studies have also documented the close association of PCOS with single-nucleotide polymorphisms (SNPs) of several genes, thereby suggesting that genetic factors are involved in its etiopathogenesis [5, 8, 9].

The fatty acid-binding proteins (FABPs) are a superfamily of proteins which play an important role in the uptake, intracellular metabolism, and excretion of long-chain fatty acids

(LCFA) [10]. Several studies have shown the linkage of SNPs of these genes with different metabolic phenotypes such as obesity, type 2 diabetes, and cardiovascular diseases, which are potential risk factors of PCOS [11]. The association of the polymorphisms of intestinal *FABP* (*FABP2*) [12, 13], liver *FABP* (*FABP1*) [14, 15], and adipose *FABP* (*FABP4*) [16] with metabolic abnormalities such as type 2 diabetes has also been demonstrated. Further, SNPs of the muscular *FABP* (*FABP3*) is reportedly associated with type 2 diabetes in the Asian population [17].

FABP1, also known as liver-type *FABP* (L-*FABP*), serves as a key regulator of lipid metabolism in the liver. The polymorphisms of *FABP1* gene have been associated with several metabolic traits. SNP rs2197076 of *FABP1* was reported to have an association with the risk of type 2 diabetes and homeostasis assessment index (HOMA-IR) [18], while SNP rs2241883 at *FABP1* was shown to be associated with an elevated risk for developing non-alcohol fatty liver disease (NAFLD).

To determine the role of *FABP1* gene in the pathogenesis of PCOS, we investigated the association of SNPs rs2197076 and rs2241883 of *FABP1* gene in women with PCOS.

Materials and methods

Study subjects

This study population comprised 221 PCOS cases (mean age 29.32 years) and 198 controls (mean age 31.43 years) from the Reproductive Hospital affiliated to Shandong University, from the period of May 2006 to Feb 2007. The eligibility criteria were in accordance with the Rotterdam consensus proposed in 2003 [2] (Rotterdam European Society of Human Reproduction and Embryology and American Society of Reproductive Medicine (ESHRE/ASRM)-sponsored PCOS consensus workshop group, 2004). The patients with PCOS were included if they met with at least two of the following three criteria: (1) chronic oligoovulation and/or anovulation; (2) clinical or biochemical hyperandrogenism; and (3) polycystic ovaries on ultrasound, ≥ 12 ovary follicles measuring 2–9 mm in diameter in one or both ovaries, or ovarian volume >10 mL [2]. PCOS patients with co-existing disorders such as congenital adrenal hyperplasia, androgen-secreting tumor, and Cushing syndrome were excluded. Care was taken to ascertain that none of the PCOS patients had undergone any hormonal treatment in the 3 months immediately prior to this test. The controls were enrolled from women who were admitted to hospital during the same period due to infertility but finally were proven healthy and infertility was attributed to fallopian tube obstruction or their husband's infertility problems such as azoospermia. The eligibility criteria for controls were (1) regular

menstrual cycle (26–35 days), (2) normal endocrine function, (3) devoid of any uterine or ovarian diseases such as polycystic ovaries on vaginal B ultrasound, and (4) no hormonal treatment in the 3 months immediately prior to the test. Women in the control group who had hyperandrogenism and hypertension were excluded.

Ethics statement

Written informed consent was obtained from all subjects. The study was approved by the Institutional Review Board at the Reproductive Hospital affiliated to Shandong University.

Measurements

Peripheral blood samples were collected from all subjects, during days 2–4 of spontaneous cycles after a 12-h overnight fast or after withdrawal bleeding. Immunoassays were performed with an automated chemiluminescent analyzer (Beckman Access Health Company, Chaska, MN, USA) to determine the levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and testosterone (T). Measurement of fasting plasma glucose (FPG) was performed photometrically using ADVIA 2400 Clinical Chemistry System (Siemens, Germany). Except for amenorrheic women, all laboratory variables were determined in the early follicular phase of the menstrual cycle.

The metabolic glucose and lipid indices were measured for women with PCOS. Both blood glucose and insulin were measured in the fasting state and 2 h post 75 g oral glucose tolerance test (OGTT), using an AU640 automatic biochemistry analyzer (Olympus Company, Hamburg, Germany). Insulin resistance was estimated by the homeostasis model assessment (HOMA-IR) method according to the formula:

$$\text{fasting glucose (mmol/L)} \times \text{fasting insulin (mIU/L)} / 22.5.$$

Fasting serum lipid profiles (serum cholesterol (CHOL), triglyceride (TG), high-density lipoprotein (HDL-C), and low-density lipoprotein (LDL-C) were measured using an enzymatic assay on an automated biochemistry analyzer (Hitachi 7150 Automatic Chemistry Analyzer; Hitachi, Tokyo, Japan). No biochemical analyses were performed for controls with normal medical conditions, and only DNA was extracted for genotyping.

Data on the following general variables were collected: height, weight, waist circumference, hip circumference, body mass index (BMI: weight [kg]/height [m²]), and waist-hip ratio (WHR: waist circumference [cm]/hip circumference). According to World Health Organization (WHO) criteria for Chinese in 1997, subjects with BMI ≥ 25 kg/m² were defined as obese [19]. Waist circumference was measured at the level

of the midpoint between the lowest rib and the iliac crest. Hip circumference was the longest measurement of the hip.

Genotyping

DNA was extracted from EDTA-anticoagulated blood by using a QIAamp DNA mini kit (QIAGEN, Hilden, Germany). The two SNPs rs2197076 and rs2241883 were analyzed by polymerase chain reaction (PCR) amplification using the following primers: rs2197076: forward 5' CTCTTGAAGACAA TGTCACCCA 3' and reverse 5' GGCTGGTTTGGATGGTCTT 3' and rs2241883: forward 5' CGCTGAGCAGAAAGGATTAGT 3' and reverse 5' CAGAGCATTGTTGTTATGAG 3'.

Polymerase chain reaction was performed on the Light Cycle system (Roche480). Reaction conditions consisted of an initial denaturation at 95 °C for 5 min followed by 35 cycles, each cycle with denaturation at 95 °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 10 min. The PCR product was sequenced by ABI Prism 3100-Avant Genetic Analyzer (Applied Biosystems), and Sequencing Analysis Software v. 4.9 was used to analyze the genotypes of SNPs.

Statistical analysis

All clinical data are expressed as means±standard deviation (SD). SPSS statistical software v. 17.0 (SPSS, Chicago, USA) was used for data analysis. Chi-square test was performed to compare allele frequencies of rs2197076 and rs2241883. Genotypes of each SNP were analyzed as additive (+/+ vs. +/- vs. -/-), dominant (+/+ plus +/- vs. -/-), and recessive (+/+ vs. +/- plus -/-). Genotype-phenotype correlation of

PCOS was analyzed by independent-sample *t* test. For phenotype analysis, chi-square test and independent *t* test were used, while logistic regression analysis was performed after adjustment for age and BMI. $P<0.05$ was considered as statistically significant.

Results

The clinical characteristics of subjects by group are shown in Table 1. All the selected clinical characteristics including age, BMI, FSH, LH, T, LH/FSH, FPG, CHOL, TG, HDL-C, and LDL-C for PCOS patients and control women were significantly different ($P<0.05$ or $P<0.001$). The mean age in the PCOS group was less as compared to that of the control group ($P<0.001$), while the BMI in the PCOS group was greater than that in the control group ($P<0.001$). Hence, age and BMI were adjusted in the subsequent analysis. Except for FPG and HDL-C, the remaining clinical parameters were significantly different after adjustment. The levels of T, LH, CHOL, TG, and LDL-C in the PCOS group were higher than those in the control group, whereas levels of FSH in the PCOS group was lower as compared to that in the control group. The results suggest that PCOS women had distinct phenotypes compared with the control women and that BMI may well stand for the central obesity indices in our subjects.

The comparison between sequence analysis of PCR products and minor allele frequency (MAF) is given in Table 2. Both rs2197076 and rs2241883 alleles of *FABP1* gene associated statistically significantly with PCOS ($P<0.001$, odds ratio (OR)=0.77; $P<0.001$, OR=0.80). Additive genotype model (rs2197076: AA/AG/GG; rs2241883: CC/CT/TT), dominant genotype model (rs2197076: AA+AG/GG;

Table 1 General clinical characteristics by study group

	PCOS (N=221)	Control (N=198)	<i>P</i>	<i>P</i> adj
Age (years)	29.320±4.164	31.430±5.585	<0.001	–
BMI (kg/m ²)	24.698±4.390	22.958±3.491	<0.001	–
FSH (IU/L)	6.347±1.504	7.624±3.343	<0.001	<0.001
LH (IU/L)	11.074±5.948	5.020±2.681	<0.001	<0.001
T (ng/dL)	45.758±21.839	25.618±8.868	<0.001	<0.001
LH/FSH	1.804±1.0271	0.713±0.384	<0.001	<0.001
FPG (mmol/L)	5.634±1.288	5.402±0.489	0.017	0.107
CHOL (mmol/L)	4.680±0.785	4.437±0.680	0.001	0.002
TG (mmol/L)	1.381±0.986	1.065±0.613	<0.001	0.027
HDL-C (mmol/L)	1.340±0.355	1.427±0.297	0.007	0.223
LDL-C (mmol/L)	3.343±0.917	2.992±0.841	<0.001	<0.001

Independent Student's *t* test and Mann-Whitney *U* test; data are expressed as mean±SD

PCOS polycystic ovary syndrome, BMI body mass index, FSH follicle-stimulating hormone, LH luteinizing hormone, T testosterone, CHOL total cholesterol, TG triglycerides, HDL-C high-density lipoprotein cholesterol, LDL-C low-density lipoprotein cholesterol, *P* adj *P* value after age and BMI adjustment in logistic regression

Table 2 Allele and genotype distributions by study group

SNP	Comparisons	PCOS minor/major	Control minor/major	<i>P</i>	Odds ratio 95 % CI	Adjustment study <i>P</i>
rs2197076 (A/G)	ALLELE	201/241	206/190	<0.001	0.77 (0.63–0.91)	<0.001
	ADD	35/131/55	38/130/30	<0.001		
	DOM	166/55	168/30	<0.001		
	REC	35/186	38/160	<0.001		
rs2241883 (C/T)	ALLELE	90/352	96/300	<0.001	0.80 (0.69–0.89)	<0.001
	ADD	10/70/141	4/88/106	<0.001		
	DOM	80/141	92/106	<0.001		
	REC	10/211	4/194	<0.001		

PCOS polycystic ovary syndrome; *ALLELE* the data of rs2197076 and rs2241883 are presented as A/G and C/T, respectively, in the PCOS and control groups; *ADD* the data was presented by the additive genotype model (rs2197076: AA/AG/GG; rs2241883: CC/CT/TT) in the two groups; *DOM* the data was presented by the dominant genotype model (rs2197076: AA+AG/GG; rs2241883: CC+CT/TT) in the two groups; *REC* the data was presented by the recessive genotype model (rs2197076: AA/AG+GG; rs2241883: CC/CT+TT) in the two groups; *Adjustment study* adjusted by body mass index in logistic regression; 95 % CI 95 % confidence interval

rs2241883: CC+CT/TT), and recessive genotype model (rs2197076: AA/AG+GG; rs2241883: CC/CT+TT) analyses showed similar results to allele comparison for two groups ($P<0.001$). After adjusting for BMI in logistic regression, there were still significant differences in the allele frequency of both SNPs between the PCOS and control groups ($P<0.001$), indicating these SNPs at *FABP1* were associated with PCOS.

To further investigate the influence of BMI, the PCOS and control subjects were subdivided into obese and non-obese groups and MAF was analyzed (Table 3). There was a significant difference in the MAF of rs2241883 of *FABP1* between PCOS and control subjects with or without obesity ($P<0.001$), and the difference retained its statistical significance even after adjusting for BMI ($P_{\text{adjusted}}<0.001$) for both the groups.

To identify the association of *FABP1* with PCOS, the genetic models of the two SNPs rs2197076 and rs2241883 of *FABP1* were analyzed using dominant and recessive models. The additive model was not performed because the statistical power was limited by a small number of homozygous minor alleles, and may have resulted in false-positive relationships. In the genotype-phenotype correlation analysis using a dominant model (Table 4),

PCOS women with the GG phenotype of rs2197076 had greater waist-hip circumference ratio (WHR) than PCOS women with AA+AG genotype ($P<0.05$). After adjusting for BMI, hip circumference (HC), WHR, and the serum levels of HDL-C in PCOS women with GG genotype were greater than those in PCOS women with AA+AG genotype ($P_{\text{adjusted}}<0.05$). In the recessive model of rs2197076 in *FABP1*, the levels of testosterone (T) in PCOS women with AA genotype were greater than those in PCOS women with AG+GG genotype. After the adjustment of BMI, PCOS women with AA genotype had greater HC, serum level of T, and ratio of LH/FSH than PCOS women with AG+GG genotype (Table 5). But for the other SNP rs2241883 of *FABP1*, in the dominant model, there was no statistical difference in all the tested features of PCOS between PCOS women with TT and PCOS women with CC+CT, and the results were the same after adjustment of BMI (Table 6). In the recessive model analysis, there was no statistical difference in all the tested features of PCOS between PCOS women with CC and PCOS women with CT+TT, whereas the serum level of HDL-C in PCOS women with CC was higher than that in CT+TT after the adjustment of BMI ($P_{\text{adjusted}}<0.05$) (Table 7).

Table 3 Allele comparisons of SNPs of *FABP1* gene in the study groups further subdivided into obese and non-obese groups

SNP	Group	MAF		<i>P</i>	OR (95 % CI)	<i>P</i> adj
		PCOS	Control			
rs2197076	Obese	85/190 (0.45)	49/92 (0.53)	0.447	0.840 (0.546–1.292)	0.089
	Non-obese	116/252 (0.46)	157/304 (0.52)	0.440	0.891 (0.665–1.194)	0.512
rs2241883	Obese	39/151 (0.26)	21/71 (0.30)	<0.001	0.87 (0.76–0.94)	<0.001
	Non-obese	51/201 (0.25)	75/229 (0.33)	<0.001	0.77 (0.65–0.86)	<0.001

P adj adjusted *P* value by BMI and age in logistic regression analysis, SNPs single-nucleotide polymorphisms, *FABP1* fatty acid-binding protein 1, PCOS polycystic ovary syndrome, MAF minor allele frequency, OR odds ratio, 95 % CI 95 % confidence interval

Table 4 The association of phenotype and genotype of rs2197076 in PCOS women by DOM genetic model

	AA+AG (N=166)	GG group (N=55)	<i>t</i>	<i>P</i>	<i>P</i> adj
BMI (kg/m ²)	24.47±4.10	25.40±5.14	1.351	0.178	–
HC (cm)	96.16±7.24	96.25±10.01	0.071	0.943	0.032
WC (cm)	85.98±11.01	88.60±13.70	1.414	0.159	0.450
WHR	0.89±0.08	0.92±0.11	2.045	0.042	0.039
FSH (IU/L)	6.43±1.55	6.91±1.34	−1.455	0.147	0.192
LH (IU/L)	11.11±5.98	10.98±5.90	−0.139	0.890	0.649
T (ng/dL)	45.57±20.94	46.31±24.53	0.217	0.828	0.394
LH/FSH	1.80±1.07	1.80±0.91	−0.011	0.991	0.606
FPG (mmol/L)	5.67±1.43	5.52±0.73	−0.754	0.451	0.280
2hPG (mmol/L)	6.77±2.60	7.07±2.05	0.781	0.435	0.719
FINS (mIU/L)	12.81±8.14	13.71±7.75	0.715	0.475	0.691
2hINS (mIU/L)	73.21±73.64	86.03±73.44	1.120	0.264	0.541
HOMA-IR	3.38±2.87	3.47±2.27	0.214	0.831	0.418
CHOL (mmol/L)	4.65±0.76	4.76±0.85	0.904	0.367	0.576
TG (mmol/L)	1.40±1.06	1.32±0.75	−0.493	0.623	0.199
HDL-C (mmol/L)	1.32±0.29	1.40±0.50	1.501	0.135	0.040
LDL-C (mmol/L)	3.30±0.89	3.46±1.00	1.123	0.263	0.469

P adj adjusted *P* value by BMI in logistic regression, *PCOS* polycystic ovary syndrome, *BMI* body mass index, *HC* hip circumference, *WC* waist circumference, *WHR* waist-hip ratio, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *T* testosterone, *FPG* fasting plasma glucose, *HOMA-IR* homeostasis assessment index, *CHOL* total cholesterol, *TG* triglycerides, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol

Table 5 The association of phenotype and genotype of rs2197076 in PCOS women by REC genetic model

	AA (N=35)	AG+GG (N=177)	<i>t</i>	<i>P</i>	<i>P</i> adj
BMI (kg/m ²)	25.00±4.38	24.66±4.42	0.417	0.677	–
HC (cm)	98.34±7.86	95.86±7.95	1.691	0.092	0.010
WC (cm)	87.57±11.91	86.33±11.68	0.571	0.568	0.614
WHR	0.89±0.07	0.90±0.08	−0.761	0.447	0.235
FSH (IU/L)	5.93±1.43	6.44±1.52	−1.834	0.068	0.074
LH (IU/L)	12.11±6.44	10.81±5.90	1.175	0.241	0.154
T (ng/dL)	55.55±29.15	42.43±17.10	2.576	0.014	0.000
LH/FSH	2.07±1.09	1.74±1.01	1.780	0.076	0.044
FPG (mmol/L)	5.50±0.63	5.68±1.41	−0.766	0.444	0.375
2hPG (mmol/L)	6.59±1.59	6.93±2.64	−0.741	0.460	0.354
FINS (mIU/L)	13.93±9.65	12.98±7.77	0.637	0.525	0.632
2hINS (mIU/L)	77.45±160.89	75.98±75.35	0.109	0.914	0.939
HOMA-IR	3.51±2.68	3.42±2.78	0.172	0.864	0.924
CHOL (mmol/L)	4.60±0.90	4.72±0.77	−0.799	0.425	0.339
TG (mmol/L)	1.37±0.73	1.40±1.05	−0.142	0.887	0.723
HDL-C (mmol/L)	1.30±0.34	1.35±0.36	−0.828	0.409	0.465
LDL-C (mmol/L)	3.23±0.82	3.42±0.92	−1.121	0.264	0.172

P adj adjusted *P* value by BMI in logistic regression, *PCOS* polycystic ovary syndrome, *BMI* body mass index, *HC* hip circumference, *WC* waist circumference, *WHR* waist-hip ratio, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *T* testosterone, *FPG* fasting plasma glucose, *HOMA-IR* homeostasis assessment index, *CHOL* total cholesterol, *TG* triglycerides, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol

Table 6 The association of phenotype and genotype of rs2241883 in PCOS women by DOM genetic model

	CC+CT (N=80)	TT (N=141)	<i>t</i>	<i>P</i>	<i>P</i> adj
BMI (kg/m ²)	24.40±4.04	24.86±4.58	0.744	0.458	–
HC (cm)	95.36±7.29	96.73±8.26	0.458	0.229	0.220
WC (cm)	85.39±10.58	87.12±12.19	1.037	0.301	0.313
WHR	0.89±0.06	0.90±0.09	0.508	0.612	0.869
FSH (IU/L)	19.62±116.03	6.40±1.50	−1.341	0.181	0.174
LH (IU/L)	11.68±6.62	10.68±5.63	−1.163	0.246	0.330
T (ng/dL)	44.98±17.19	44.34±21.54	−0.220	0.826	0.766
LH/FSH	1.94±1.20	1.71±0.92	−1.575	0.117	0.155
FPG (mmol/L)	5.52±0.76	5.72±1.52	1.077	0.283	0.356
2hPG (mmol/L)	6.79±1.86	6.91±2.79	0.333	0.740	0.926
FINS (mIU/L)	12.60±6.84	13.12±8.90	0.442	0.659	0.947
2hINS (mIU/L)	76.54±62.06	75.52±78.26	−0.098	0.922	0.646
HOMA-IR	3.18±1.95	3.51±3.15	0.837	0.404	0.624
CHOL (mmol/L)	4.69±0.75	4.69±0.81	0.055	0.956	0.869
TG (mmol/L)	1.30±0.83	1.43±1.08	0.883	0.378	0.534
HDL-C (mmol/L)	1.37±0.40	1.33±0.33	−0.900	0.369	0.483
LDL-C (mmol/L)	3.38±0.96	3.37±0.88	−0.101	0.920	0.687

P adj adjusted *P* value by BMI in logistic regression, *PCOS* polycystic ovary syndrome, *BMI* body mass index, *HC* hip circumference, *WC* waist circumference, *WHR* waist-hip ratio, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *T* testosterone, *FPG* fasting plasma glucose, *HOMA-IR* homeostasis assessment index, *CHOL* total cholesterol, *TG* triglycerides, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol

Table 7 The association of phenotype and genotype of rs2241883 in PCOS women by REC genetic model

	CC (N=10)	CT+TT (N=202)	<i>t</i>	<i>P</i>	<i>P</i> adj
BMI (kg/m ²)	23.81±3.50	24.76±4.45	−0.659	0.510	
HC (cm)	94.70±7.06	96.35±8.02	−0.637	0.525	0.905
WC (cm)	83.10±8.89	86.71±11.82	−0.952	0.342	0.435
WHR	0.88±0.07	0.90±0.08	−0.813	0.417	0.602
FSH (IU/L)	5.62±0.85	11.35±70.70	−0.256	0.798	0.816
LH (IU/L)	10.57±5.14	11.05±6.05	−0.244	0.807	0.600
T (ng/dL)	41.50±18.10	44.75±20.24	−0.499	0.618	0.668
LH/FSH	1.88±0.78	1.78±1.04	0.291	0.772	0.937
FPG (mmol/L)	5.45±0.63	5.66±1.34	−0.493	0.623	0.730
2hPG (mmol/L)	7.33±1.88	6.85±2.53	0.586	0.558	0.396
FINS (mIU/L)	10.39±4.14	13.13±8.36	−1.025	0.307	0.435
2hINS (mIU/L)	74.20±39.34	76.32±74.36	−0.090	0.929	0.832
HOMA-IR	2.56±1.12	3.45±2.84	−0.986	0.325	0.462
CHOL (mmol/L)	4.94±0.97	4.69±0.78	0.979	0.329	0.224
TG (mmol/L)	1.31±0.70	1.39±1.02	−0.253	0.801	0.974
HDL-C (mmol/L)	1.64±0.73	1.33±0.33	1.320	0.219	0.011
LDL-C (mmol/L)	3.58±1.44	3.38±0.88	0.442	0.669	0.315

P adj adjusted *P* value by BMI in logistic regression, *PCOS* polycystic ovary syndrome, *BMI* body mass index, *HC* hip circumference, *WC* waist circumference, *WHR* waist-hip ratio, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *T* testosterone, *FPG* fasting plasma glucose, *HOMA-IR* homeostasis assessment index, *CHOL* total cholesterol, *TG* triglycerides, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol

Discussion

Polycystic ovary syndrome is characterized by oligomenorrhea or amenorrhea, hyperandrogenism, and polycystic ovaries [2]. Women with PCOS generally have some common characteristics like menstrual irregularity, obesity, subfertility, acne, hirsutism, and abnormal biochemistry accompanied with raised serum testosterone, androstenedione, insulin, and luteinizing hormone levels [20]. PCOS is a heterogeneous disorder with a complex and multifactorial etiology. Obesity and associated metabolic alterations such as insulin resistance play an important role in the development and maintenance of PCOS pathology at least in a substantial proportion of patients [21]. In recent years, numerous studies have suggested a role of genetic factors in the etiology of PCOS [22–24]. Discovery of SNP as new markers of the human genome opens a novel way to investigate the genetic association of candidate genes in the pathogenesis of complex disorders including PCOS. A number of functional SNPs of certain genes have been demonstrated to be associated with PCOS [25, 26].

The liver fatty acid-binding proteins, a member of the superfamily of the FABPs, are found in abundance in the cytosol of liver parenchymal cells [27]. As a key regulator of hepatic lipid metabolism, FABP1 influences fatty acid uptake, trafficking, mitochondrial oxidation, and esterification [28, 29]. FABP1 is also involved in the ligand-dependent transactivation of peroxisome proliferator-activated receptor α (PPAR α) in translocating long-chain fatty acids to the nucleus [30]. Peroxisome proliferator-activated receptor α regulates the transcription of several genes involved in lipid metabolism [31, 32]. PPAR α -deficient mice showed a propensity for development of massive hepatic lipid accumulation, hypercholesterolemia, and hypertriglyceridemia [33]. SNP rs2241883 variant T/C in exon 3, resulting in a threonine (T) to alanine (A) substitution in the encoded protein (T94A), was initially identified by Brouillette et al. in 2004 [14]. This common SNP rs2241883 variant in the human *FABP1* gene has been shown to have an important role in hepatic lipid metabolism and is associated with elevated cholesterol and triacylglycerol levels, which in turn are associated with an increased risk of cardiovascular disease, type 2 diabetes, and metabolic syndrome [14, 34]. The polymorphisms of *FABP1* gene have been associated with several metabolic traits. In an association study regarding SNPs of *FABP1* and non-alcohol fatty liver disease in a Chinese population, SNP rs2241883 of *FABP1* was associated with an increased risk for developing non-alcohol fatty liver, and rs2241883 carriers had a higher level of LDL-C. However, the exact role of *FABP1* T94A polymorphism in non-alcohol fatty liver remains elusive. Given that the T94A mutation exhibits a lower binding capacity of FABP1 for LCFA [31], this may result in reduced activation of PPAR α by lower FABP1-

bound LCFAs in *FABP1* rs2241883 C allele carriers, which results in inhibition of expression of genes involved in lipid metabolism, contributing to non-alcohol fatty disease. Among SNPs of *FABP1*, another SNP rs2197076 has been shown to have a strong association with the risk of type 2 diabetes and HOMA-IR.

SNPs rs2197076 and rs2241883 of *FABP1* could affect hepatic lipid accumulation and result in altered lipid metabolism thereby making patients prone to type 2 diabetes or insulin resistance, which are highly associated with PCOS. In the present study, we analyzed the association of SNPs rs2197076 and rs2241883 of *FABP1* with PCOS. With a cohort of 221 PCOS cases and 198 controls, allele frequency analysis showed that both SNPs of *FABP1* had a strong association with PCOS ($P < 0.001$, OR = 0.77; $P < 0.001$, OR = 0.80). Their additive, dominant, and recessive genotype model analyses further supported this association, even after adjusting for age and BMI. The BMI in PCOS women was significantly greater than that in the control cohort, indicating that the association of *FABP1* and PCOS has been influenced by BMI to a certain extent. Owing to the genetic impact of *FABP1* on obesity, we stratified all subjects by obesity to analyze the association of these SNPs with PCOS. MAF of rs2241883 in obese PCOS women was less than that in obese control women, but MAF in non-obese PCOS women was still less as compared to that in non-obese control women, indicating a direct effect of both alleles of *FABP1* in causing PCOS, regardless of obesity or BMI.

Further, we used genotype-phenotype correlation analysis to investigate the contributions of rs2197076 and rs2241883 of *FABP1* to some clinical or biochemical features of PCOS. We observed contrasting results for both SNP rs2197076 and rs2241883 of *FABP1* gene. Both dominant and recessive models showed statistically significant association with biochemical parameters with and without BMI adjustments, while rs2241883 showed no association in the dominant model and showed minimal association with biochemical parameters with BMI adjustments.

These results indicate that although both SNP rs2197076 and rs2241883 of *FABP1* gene were associated with PCOS, they had different contributions to the pathogenesis of PCOS. Due to its close relationship to some important clinical features of PCOS, allele rs2197076 seemed to play a more important role in the mechanism of PCOS than allele rs2241883. SNP rs2241883 leads to Thr94Ala amino acid change that is located within the N-terminal region of the protein, a component of the fatty acid binding site. SNP rs2197076 may regulate the functional activity of the protein, individually or in haplotypes, because of its location in the 3' UTR site, and its association with a potential functional variant in one risk haplotype [18]. The different forms of FABP1 protein due to genetic differences in the two SNPs may have varied contributions in the causation of PCOS. The detailed

association of *FABP1* and etiology of PCOS needs further evaluation in a larger study group.

There are several limitations in the present study. The sample size in PCOS and control women was relatively small, which limits the statistical power of the analyses. Hence, validation of our findings is required in studies with a larger sample. Secondly, we did not test the association of *FABP1* by categorizing PCOS women into diabetes and insulin resistance with PCOS and hyperandrogenism with PCOS owing to the small sample size because *FABP1* could be potentially associated with a specific subtype of PCOS.

In conclusion, the current study lends new insights into the role of *FABP1* gene in the pathogenesis of PCOS. We found two SNPs rs2197076 and rs2241883 of *FABP1* gene to be associated with PCOS. SNP rs2197076 was related with some important clinical features of PCOS and seemed to play a more important role in the mechanism of PCOS than SNP rs2241883. To the best of our knowledge, this is the first study to provide evidence of an association between *FABP1* genetic variants and PCOS women.

Acknowledgments This work was supported by the National Basic Research Program of China (973 program) (2012CB944700 and 2011CB944502) and the Science Research Foundation Item of No-Earnings Health Vocation (201002013). We also acknowledge Li You, Yuehong Bian, Changming Zhang, Guangyu Li, and Yongzhi Cao for specimen collection and technology support.

Compliance with ethical standards

Ethics statement Written informed consent was obtained from all subjects. The study was approved by the Institutional Review Board at the Reproductive Hospital affiliated to Shandong University.

Competing interests The authors declare that they have no competing interests.

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