Common Variant rs9939609 in Gene FTO Confers Risk to Polycystic Ovary Syndrome

Tao Li[1,2◊], Keliang Wu[1,2,3,4◊], Li You[1,2,3,4◊], Xiuye Xing[1,2], Peng Wang[1,2], Linlin Cui[1,2], Hongbin Liu[1,2,3,4], Yuqian Cui[5], Yuehong Bian[1,2,3,4], Yunna Ning[1,2,3,4], Han Zhao[1,2,3,4], Rong Tang[1,2]*, Zi-Jiang Chen[1,2,3,4,5]*

1 Center for Reproductive Medicine, Provincial Hospital Affiliated to Shandong University, Jinan, China, 2 National Research Center for Assisted Reproductive Technology and Reproductive Genetics, Jinan, China, 3 The Key Laboratory for Reproductive Endocrinology of Ministry of Education, Jinan, China, 4 Shandong Provincial Key Laboratory of Reproductive Medicine, Jinan, China, 5 Department of Obstetrics and Gynecology, Renji Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai, China

Abstract

Background: Fat mass and obesity-associated gene (*FTO*) has been associated with obesity, especially the common variant rs9939609. Polycystic ovary syndrome (PCOS) is a complex endocrine-metabolic disorder and over 50% of patients are overweight/obese. Thus *FTO* is a potential candidate gene for PCOS but their relationship is confusing and remains to be clarified in different population with a large sample size.

Method: This study was performed adopting a two-stage design by genotyping SNP rs9939609. The first set comprise of 741 PCOS and 704 control subjects, with data from our previous GWAS. The second phase of replication study was performed among another independent group of 2858 PCOS and 2358 control subjects using TaqMan-MGB probe assay. All subjects are from Han Chinese.

Results: The less meaningful association of *FTO* rs9939609 and PCOS discovered in GWAS (P = 2.47E-03), was further confirmed in the replication study (P = 1.86E-09). Using meta-analysis, the P-meta value has reached 6.89E-12, over-exceeding the genome-wide association level of 5.00E-8. By combination, the P value was 1.26E-11 and after BMI adjustment it remained significant(P = 1.82E-06). To further elucidate whether this association is resulted from obesity or PCOS per se, the samples were divided into two groups–obese and non-obese PCOS, and the results were still positive in obese group (P obese = 5.81E-05, OR = 1.55), as well as in non-obese PCOS group (P non-obese = 7.06E-04, OR = 1.28).

Conclusion: Variant rs9939609 in *FTO* is associated with PCOS in Chinese women, not only in obese PCOS subjects, but also in non-obese cases.

Introduction

Polycystic ovary syndrome (PCOS) is a complex endocrine-metabolic disease affecting 6–8% women of reproductive age [1]. Characterized by clinical and/or biochemical androgen excess, ovulatory dysfunction and polycystic ovaries, PCOS patients is also associated with an increased risk of overweight/obesity, insulin resistance and type 2 diabetes mellitus (T2DM) [1,2,3,4]. Over 50% PCOS cases are overweight/obese [5]. Evidence implies obesity, interacting with T2DM and endocrine disorders, is an important factor in the etiology of PCOS [6,7]. And the prevention and treatment of obesity will benefit PCOS patients [8]. Family-based and case-control association studies suggest genetic factors contribute to both obesity and PCOS, which

implicates a shared genetic predisposition in their concurrence [9,10,11].

Fat mass and obesity associated gene (*FTO*), located on chromosome 16q12.2, is expressed in a wide range of tissues including adipose tissue, muscle, pancreas, and liver, with the highest concentrations in the hypothalamus [12]. *FTO* has been wildly identified to be associated with body mass index (BMI) and obesity by large scale genome-wide association studies and a wealth of replication studies in several ethnicities, including Chinese [13,14,15,16,17,18]. Besides obesity, *FTO* also confers risk to T2DM [19,20,21], although this association may be independent of BMI in East and South Asian [22]. In above studies common variant rs9939609 is representative SNP marker. Considering both obesity and T2DM are major complications of PCOS, *FTO* may also confer risk to PCOS.

The association of *FTO* and PCOS has been widely investigated, but the results remain controversial. Variant rs9939609 of *FTO* was proved to be associated with PCOS in U.K. women [23], while the association was not verified in American and European Caucasian [24,25]. In Chinese this result has been replicated but became not significant after adjustment by BMI [26]. All of these studies have been characterized by a limited sample size and statistical power. Therefore, enlarged sample size is requested to get a credible interpretation.

The aim of this study was to investigate the relationship between *FTO* variant and PCOS whether on the effect of BMI. After reviewing our previous discovery cohort of GWAS (Table S1 in file S1) [27], we conducted a replication study. A total of 3599 PCOS cases and 3082 control subjects were enrolled. Obesity-related traits such as BMI, waist–hip ratio, glucose and lipid profiles in different genotypes of rs9939609 were also analyzed in PCOS patients.

Materials and Methods

Subjects

Based on the Rotterdam Consensus proposed in 2003 [28], diagnosis of PCOS were determined as two of the following characteristics existence at least, oligo−/aovulation (OA), polycystic ovarian morphology (PCO), clinical or biochemical hyperandrogenism (HA). The diagnosis of PCOS was made only when the other etiologies for hyperandrogenemia and ovulatory dysfunction were excluded, i.e. congenital adrenal hyperplasia, 21-hydroxylase deficiency, androgen-secreting tumors, Cushing's syndrome, thyroid disease, and hyperprolactinemia. A total of 3599 women with PCOS, among which 741 were originated from our first GWAS and 2858 from the independent replication study, were recruited at the reproductive medical center of Shandong Provincial Hospital affiliated to Shandong University from Jun 2009 to May 2012.

A total of 3082 age-matched healthy women, comprising of our first GWAS (704) and the replication study (2378), were enrolled of the same time period. All subjects in control group had regular menstrual cycle (26–35 days) and normal ovarian morphology. Total testosterone and modified Ferriman-Gallwey score were evaluated for exclusion of hyperandrogenism.

All of participants were unrelated individuals of reproductive age with no hormonal therapy for at least three months prior to the test. Medication history and history of weight loss for all subjects were collected to exclude these who have had weight or glucose affecting measures such as those taking metformin or any other oral hypoglycemic agents.

Ethics Statement

The study was approved by the Institutional Review Board of Reproductive Medicine of Shandong University. All participants were informed of the genetic detection and written informed consent was obtained.

Clinical and Biochemical Measurements

Height and weight were measured with participants dressed in underwear without shoes. BMI (kg/m^2) was calculated as the weight in kilograms divided by the square of height in meters. Obesity was defined as BMI≥25 kg/m^2 according to 1997 WHO criteria for Chinese [29]. Waist circumference was measured midway between the lowest rib and the iliac crest. Hip circumference was the longest measurement of hip. Waist-hip ratio (WHR) was defied as waist circumference divided by hip circumference.

Peripheral blood samples were collected during day 2–4 of spontaneous cycles or after withdrawal of bleeding from all subjects after a 12-h overnight fast. Follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, testosterone (T) and estradiol (E2) of all subjects were measured by a chemiluminescent analyzer (Beckman Access Health Company, Chaska, MN, USA).

Glucose Metabolic and Lipid Measurements

The glucose metabolic and lipid indices were measured for woman with PCOS. Blood glucose and insulin of fasting and 2 hours after a 75-g oral glucose tolerance test (OGTT) was measured (AU640 automatic biochemistry analyzer; Olympus Company, Hamburg, Germany.). Insulin resistance was assessed using the homeostasis model (HOMA-IR = fasting blood glucose (FBG mmol/L)×Fasting insulin (FINS mIU/L)/22.5). Serum cholesterol (CHOL), triglycerides (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL) were evaluated by enzymatic method. Of the controls whose medical conditions were normal by body examination in our department, we only extracted DNA for genotyping.

Table 1. Basic clinical characteristics comparisons between PCOS and Control subjects.

Characteristics	Groups (Mean±SD)		P	P adj
	PCOS (3599)	CONTROL (3082)		
AGE (years)	28.35±3.75	31.33±4.69	<0.001	–
BMI (kg/m^2)	24.81±4.29	22.73±3.15	<0.001	–
WAIST (cm)	84.14±11.10	77.80±7.69	<0.001	0.13
WHR	0.87±0.06	0.84±0.05	<0.001	0.9
FSH (IU/L)	6.30±1.90	7.30±3.02	<0.001	<0.001
LH (IU/L)	10.38±5.81	4.89±2.37	<0.001	<0.001
T (ng/dl)	55.51±24.83	21.53±12.66	<0.001	<0.001

SD = standard error. WHR = wasit-hip ratio. FSH = follicle-stimulating hormone. LH = luteinizing hormone. T = testosterone. P adj, adjusted P value by age and BMI in logistic regression.

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Table 2. Allele comparison of rs9939609 in GWAS and replication study.

	Risk Allele	MAF PCOS	MAF control	P	ORs 95% CI	P adj	P meta
GWAS	A	0.131	0.095	**2.47E-03**	1.43	**2.30E-02**	**6.89E-12**
					1.13–1.81		
Replication study	A	0.141	0.102	**1.86E-09**	1.44	**4.00E-04**	
					1.28–1.68		

GWAS = genome-wide association study. In GWAS study, PCOS: control = 741:704. In replication study, PCOS: control = 2858:2378. MAF = minor allele frequency. ORs = Odds Ratios. 95% CI = confidence interval. P adj, adjusted P value by BMI and age in logistic regression. P meta, meta-analysis of GWAS and replication study by PLINK.
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SNP Genotyping

Genomic DNA was extracted with QIAamp DNA mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. Subjects of GWAS were genotyped by Affymetrix Genome-Wide Human SNP Array 6.0 [27]. For the replication study, variant rs9939609 was analyzed by TaqMan-MGB probe assay (Invitrogen trading, Shanghai) by available primers and probes. The reactions were performed on 384-well plates, Roche Lightcycle 480 carried out by pre-incubation at 95°C for 4 min followed by 45 cycles of denaturation at 95°C for 15 s, annealing, extension and detection for 40 s at 60°C. 100 PCOS and 100 control participants from the GWAS cohort were re-tested to make sure the coordinance of different methods. Direct sequencing of 5% randomly selected samples were applied to validate the genotyping assays. And the success rate was 99.5%.

Statistical Analysis

Continuous parameters of patients and controls are expressed as means±SD. Genetic Power Calculator [30] was applied for the sample size estimation and case-control genetic power calculation. PLINK (v.1.07, http://pngu.mgh.harvard.edu/purcell/plink) was applied to calculate the Hardy-Weinberg equilibrium (P), allele frequency differences and genotype differences. Odd ratios (ORs) with 95% CIs (confidence intervals) are presented. Meta-analysis for the all samples was performed by the fixed effects Cochran-Mantel-Haenszel (CMH) test by PLINK. Genetic models were divided into additive (+/+ vs. +/− vs. −/−), dominant (+/+ plus +/− vs. −/−) and recessive (+/+ vs. +/− plus −/−). By the additive effects of allele dosage, the logistic regression for disease trait was conducted to exclude the potential confounding effects of age and/or body mass index. The genotype models were applied for the phenotype analysis which was compared by one-way

ANOVA or student T test, SPSS, 16.0 (SPSS Inc., Chicago, IL, USA). And P<0.05 was regarded as statistically different.

Results

Basic Clinical Measurements

3599 PCOS patients and 3082 controls were recruited and the case-control genetic power has reached 0.8 at P = 1E-5 level. Basic clinical measurements were compared and presented in Table 1.

In PCOS group, 44% (1577/3587) subjects were obese (BMI≥25 kg/m^2) while the percentage in control was 21.4% (660/3082). PCOS women had significantly higher levels of luteinizing hormone and testosterone. In logistic regression, the differences of waist circumference and WHR were not significant after age and BMI adjustment (P $_{wasit}$ = 0.13, P $_{WHR}$ = 0.9), which implies BMI may well stand for the central obesity indices in our study subjects.

Allele and Genotypes

Data of our study were consisted of two parts that were GWAS data (741 PCOS cases vs. 704 control subjects) and replication study (2858 PCOS case vs. 2378 control subjects). The observed genotype distribution was in consistent with Hardy–Weinberg equilibrium in both GWAS and replication study respectively. Minor allele frequency (MAF) comparison was presented in Table 2. In the two parts, the association of rs9939609 and PCOS has been discovered (P $_{GWAS}$ = 2.47E-03, OR = 1.43; P $_{replication}$ = 1.86E-09, OR = 1.44). The P value of meta-analysis is 6.89E-12 (I^2 = 92%), which has reached the GWAS standard of significance (P<5.0E-8).

In order to eliminate the function of BMI in the association, the two cohorts were combined and analyzed (Table 3). After

Table 3. Allele and genotype analysis of combined data.

SNP	Comparisons	PCOS	control	P	OR	Adjustment study	
		Minor/Major	Minor/Major		95%CI	STAT	P
rs9939609 A/T	ALLELE	1001/6197	621/5543	**1.26E-11**	1.44	4.77	**1.82E-06**
					1.30–1.60		
	ADD	67/867/2665	29/563/2490	**1.09E-10**	–	–	–
	DOM	934/2665	592/2490	**5.95E-11**	–	–	–
	REC	67/3532	29/3053	**1.62E-3**	–	–	–

Allele, the data was presented as A/T in the two groups. ADD, the data was presented by the additive genotype model (AA/AT/TT) in the three groups. DOM, the data was presented by the dominant genotype model (AA+AT/TT) in the two groups. REC, the data was presented by the recessive genotype model (AA/AT+TT) in the two groups. Adjustment study, adjusted by BMI in logistic regression.
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Table 4. Allele comparisons of PCOS and control divided into obese and non-obese group.

	MAF		P	OR	P adj
	PCOS	control		95% CI	
Obese group	0.163	0.114	5.81E-05	1.55 (1.25–1.92)	4.43E-05
Non-obese group	0.121	0.097	7.06E-04	1.28 (1.11–1.48)	2.74E-04

In obese group, PCOS: control = 1577:660. In non-obese group, PCOS: control = 2010:2422. P adj, adjusted P value by BMI and age in logistic regression. MAF = minor allele frequency. OR = odds risk. 95% CI = confidence interval.
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adjustment of BMI, the difference between PCOS and control was still significant (P adjusted = 1.82E-06). The OR of combined study was similar to the above (P combined = 1.26E-11, OR = 1.44).

To further illuminate the influence of BMI, the PCOS subjects were divided into obese group and non-obese groups. Compared with BMI matched controls, in obese PCOS group, MAF was higher than BMI matched controls (P_{obese} = 5.81E-05, OR = 1.55; Table 4), and the difference was still dominant after age and BMI adjustment ($P_{adjusted}$ = 4.43E-05).

Similarly in the non-obese PCOS, the the $P_{non-obese}$ value was 7.06E-04 (OR = 1.28). And the difference in non-obese subjects was even more evident after BMI and age adjustment (non-obese group, $P_{adjusted}$ = 2.74E-04).

Clinical and Metabolic Measurements in PCOS

There was a gradient in weight and BMI with the highest in homozygous A allele individuals and the lowest in individuals with homozygous T allele in PCOS (1.53 kg increase in weight, P = 3.6E-05; 0.76 kg/m2 increase in BMI, P = 1.7E-04). Apparently, BMI and waist circumference increased along with increase of the risk allele A (Table S2 in file S1). However, there were no other differences exiting in the parameters of endocrine and metabolic characteristics including glucose metabolic indices and lipid profiles (Table S3 in file S1).

Discussion

Polycystic ovary syndrome, as well as individual susceptibility to obesity, is thought to be caused by interactions between genetic make-up and the environment. Recently, GWASs on PCOS have identified a series of susceptibility loci, whereas few are obesity related [27,31]. If PCOS and obesity share a common genetic background, replicating the obesity susceptibility genes in PCOS patients is a helpful method to discover risk variants, which needs well exclusion of confounding factors. FTO is a common candidate gene for obesity and BMI [17]and probably contribute to PCOS. In this study, we recruited a large number of samples and identified that FTO is the susceptibility gene for PCOS regardless of the presence of obesity.

In our study, with a cohort of total 3599 PCOS cases and 3083 controls, we identified strong evidence that risk allele A of rs9939609 confers higher risk to PCOS ($P_{combined}$ = 1.26E-11, ORs = 1.44; P_{meta} = 6.89E-12), even after BMI adjustment (P = 1.82E-6). SNP rs9939609 has been the common variant in the identification of FTO as the susceptibility gene to obesity and T2DM [13,14,15,16,17,18,19,20,22]. And the genotype of rs9939609 would influence FTO expression in transcript level [32,33] and thus is related to fat cell lipolysis [34]. Studies also proved that rs9939609 related to increased food responsiveness and better emotional control [35]. As the association has been identified in our study, functional studies about rs9939609 in PCOS would help to the explain the etiology.

The relationship of FTO and PCOS has been investigated, but the association has been influenced by BMI to a large extent. As concluded in Table 5, the association of FTO with PCOS susceptibility has not been observed in Caucasian and Korean women [24,25,36,37]. Even the association has been discovered in studies of Barber et al. and Yan et al., it became nonsense in obese PCOS by BMI stratification analysis, indicating that the predom-

Table 5. Previous association studies of FTO and PCOS.

	SNP	Population	Sample size	MAF(%)		P	Adjustment study	BMI-stratified study	
			PCOS/CTRL	PCOS	CTRL		P	High-BMI	Low-BMI
Barber et al. (22)	rs9939609	Caucasian	463/1336	46.3	40.0	7.4E-04	2.9E-03	2.9E-04	0.11
Attaoua et al. (31)	rs1421085	Caucasian	207/100	49	46	>0.05	>0.05	<0.04	>0.05
Yan et al (25).	rs9939609	Chinese	215/227	15.1	9.9	0.019	0.286	0.016	0.065
Saxena et al. (24)	rs9939609	Caucasian and African	510/448	43.1	39.6	0.811	1	–	–
Ewens et al.(23)	rs9939609	Caucasian	439 trios 395/176	45.6; –	-	0.575; –	–	–	–
Kim et al. (32)	rs1421085	Korean	377/386	15.7	15.2	0.789	–	–	–
Current study	rs9939609	Chinese	3599/3082	13.9	10.1	1.26E-11	1.82E-06	5.81E-05	7.06E-04

CTRL = control; MAF = minor allele frequency.
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inant of *FTO* on PCOS susceptibility mainly mediated through BMI [23,26]. As *FTO* has been identified to be susceptibility gene to obesity, the entanglement of obesity and PCOS suggests that adjustment analysis by BMI would not be as effective. It is necessary to stratify all participants by obesity to eliminate the bias due to the genetic effect of the rs9939609 SNP with obesity. In our study the strong association was some extent impacted by BMI, but still evident in non-obese PCOS (P = 2.74E-04), as well as in obese group (P = 4.43E-05). As rs9939609 has been the representative variant of *FTO*, a direct effect of *FTO* conferred to PCOS has been revealed regardless of obesity or BMI.

FTO, as confirmed by our study, confers risk to the pathogenesis of PCOS per se; at the meanwhile, increased BMI may have synergetic effect on PCOS by *FTO* function. Obesity is associated with hyperandrogenism and menstrual disturbance of PCOS and worsens PCOS complications such as T2DM [4,7,22], we hypothesize that *FTO* should be one of the molecular determinants linking obesity to PCOS. *FTO* is associated with eating habit and is important to the control of energy homeostasis [38]. It acts as a transcriptional coactivator in epigenetic regulatory processes [39] and nucleic acid repair or modification processes [40], which is important in modulating the hyperandrogenism and involves in the ovarian dysfunction of PCOS [41]. The role of *FTO* in general mechanism and epigenetic regulation suggests that *FTO* may be a pleiotropic factor involved in various diseases such as PCOS, obesity and T2DM. However, the function and biological pathways of *FTO* protein in PCOS has not been fully considered. Further studies on *FTO* in PCOS patients will provide possible interpretation for its etiology and interaction of obesity and PCOS.

Clinical and metabolic measurements have been analyzed for the understanding of *FTO* effects in PCOS. Different from the study of Yan et al [26], our study confirmed the genotype-dependent relationship between rs9939609 risk allele and BMI of PCOS, which has also been revealed in the studies and meta-analysis of the Caucasian cases [23,42,43]. Interestingly, it was not found in control group yet. The increases of BMI and weight, similar to Tan's report [42], were not as high as the meta-analysis of eight distinct cohorts by Wojciechowski et al. [43]. Considering

the meta-analysis did not cover Asian patients, it may due to the different genetic backgrounds between ethnicities. Although Wehr et al. [44] demonstrated an increased hyperandrogenemia indices in PCOS group along with rs9939609, it was not shown in our study. The reason may due to the difference that hyperandrogenism is less prevalent in Chinese Han than Caucasians. As to other metabolic profiles, no significance was found in our study, nor other previous studies [45]. However, subjects enrolled in the study are reproductive-aged young people and few of them suffered from metabolic disorders. More profound researches are expected to learn the pathophysiological effect of *FTO* in PCOS and tracking PCOS women would help to clarify the relationship of *FTO* and metabolic disturbances.

In summary, we have demonstrated that variant rs9939609 in *FTO* is associated with PCOS in Chinese women. The association has been disclosed both in obese group and non-obese group. Nonetheless, more in-depth studies are required to elucidate the biological role of *FTO* playing on the interaction of obesity and PCOS.

Supporting Information

File S1.
(DOC)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: HZ TL. Performed the experiments: TL YC YB YN. Analyzed the data: TL. Contributed reagents/materials/analysis tools: KW LY HL. Wrote the paper: TL LC XX. Edited the manuscript: HZ PW RT. Final approval of the version to be published: RT Z-JC.

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